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EXOGENOUS APPLICATION OF PHYTOHORMONES MODULATES THE ANTIOXIDANT ENZYME SYSTEM TO INDUCE TERMINAL HEAT STRESS TOLERANCE IN CHICKPEA

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Abstract

Heat stress has been considered as vital ecological feature to affect the plant growth and productivity. Rising temperature due to global warming resulting in heat stress that restricting the plant growth and potential productivity throughout the world. Therefore, a field experiment was carried out at Bahauddin Zakariya University Multan, Pakistan to minimize the heat stress induced-losses in chickpea plants with the foliar spray of phytohormones during two consecutive years 2017-18 and 2018-19. Experimental treatments comprising of two chickpea cultivars (DHUST and Bhakkar, 2011) were cultivated on two different sowing dates (15th November and 1st December) and foliar spray of phytohormones (Benzyl-amino purine (BAP) and Moringa leaf extract (MLE30)) including tap water as a control. Results suggested that heat 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 significantly modulated superoxide dismutase, peroxidase and catalase enzymatic activities and minimized the adverse impacts of heat stress on yield contributing traits. Our results suggested that foliar spray of phytohormones particularly MLE modulated the heat stress induced losses to chickpea cultivar Bhakkar 2011 by improving their antioxidant defense mechanism and enhanced the productivity.

Key words: Benzyl-amino purine, Catalase, Seed yield, Moringa leaf extract, Peroxidase

Introduction

Chickpea (*Cicer arietinum* L.) is one of leading leguminous crop, plays a vital part in nourishment for huge population of the undeveloped states and is ranked as a healthy food in several well-developed countries (Merga & Haji, 2019). It also fixes atmospheric nitrogen and an essential source of forage, therefore plays a major role in improving the economic return of the present cropping systems therefore occupies an essential position in crop rotations in the world (Khaitov *et al*., 2016). Although chickpea is a winter season crop, however, heat stress at later stage of reproductive growth often reduce its productivity. Though, environment changes increased the average temperature around 0.74°C per 100 years (Anon., 2007) however, during the last 50 years, the linear heating tendency has been approximately twofold the rate of the preceding 100 years. To appraise that the increase in worldwide average temperature between 1.8 and 4°C at the end of 21st century, depending on the greenhouse emissions and disparities in precipitation patterns (Anon., 2007). These variations in temperature will definitely influence crop growth and reduce productivity up to 30% (ICRISAT, 2009).

Unfavorable influences of abiotic stresses on chickpea plants can be minimized with the foliar spray of bio-stimulants and synthetic growth promoters that bring significant variations in plant physiology and biochemistry (Verma *et al*., 2016). Among various growth promoters, phytohormones such as benzyl-amino purine (BAP) and moringa leaf extract (MLE) possess a definite role in stimulating abiotic stress tolerance by generating modulations at molecular levels (Abd El-Rahman & Mohamed, 2014). Foliar spray of these phytohormones 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 & Dawood, 2014).

Though lot of research has been conducted on distinctive crops to alleviate the adverse influences of heat stress by foliar spray of phytohormones. However, only a few 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 to improve the chickpea production in heat stress environments. Keeping in view the aforementioned discussion, the existing two years study was designed to explore the influence of heat stress and role of natural and synthetic growth promoters in mitigating its adverse effects on chickpea.

Materials and Methods

A field experiment was performed at Research area of Bahauddin Zakariya University, Multan, Pakistan to estimate the adverse effects of heat and stimulate heat stress tolerance in chickpea with exogenous application of phytohormones during two consecutive years 2017-18 and 2018-19. The experiment was designed in completely randomized blocks with split plot arrangements and had three repeats. Experimental treatments comprised of two chickpea cultivars (DHUSHT and Bhakkar, 2011), two sowing dates (15th November and 1st December) and two foliar spray of phytohormones (moringa leaf extract (MLE30) (Arif *et al*., 2019), and Benzyl-amino purine (BAP) 50 mg L^{-1} (Ali *et al*., 2011) including tap water as a control. Chickpea plants were sprayed twice on 16th January and 15th February by using hand sprayer during both growing seasons. The water spray treatment was applied first and the volume of water to be used was determined. Then in other two treatments having same volume of water with MLE30 1:29 (Arif *et al.*, 2019) and BAP 50 mg/L were sprayed (Ali *et al.*, 2011).

Preparation of moringa leaf extract (MLE): Moringa leaves are potential source of zeatin that enhances the antioxidant properties of many enzymes and protects the cells from injuries. Young leaves of *Moringa oleifera* were harvested and washed several times with distilled water then stored in freezer at -5°C for 12 hours. Moringa frozen leaves were crushed in a juicer for extraction according to the procedure explained by Yasmeen *et al.*, (2018). The extract was filtered twice by using Whatman No.1 filter paper and then centrifuged at 8000 g for 20 minutes and diluted 30 times with distilled water. Using different methodologies, moringa leaf extract (MLE) was analyzed for chemical composition. Eighteen chemical constituents were identified in the moringa leaf extract; they are total soluble protein (1.40 mg g^{-1}), super oxide dismutase (191.86 IU min^{-1} mg $protein^{-1}$), peroxidase (21.99 mmol min^{-1} mg $protein^{-1}$), catalase (7.09 mmol mi n^{-1} mg $protein^{-1}$), total phenolic contents (8.19 mg g^{-1}), ascorbic acid (0.36 m mole g^{-1}), gibberellins (0.74 mg g^{-1}), zeatin (0.96 mg g^{-1}), nitrogen (1.93%), phosphorus (0.18%), Potassium (2.19%), Calcium (2.43%), Magnesium (0.012%), Zinc (38.33 mg k g^{-1}), Copper (3.50 mg k g^{-1}), iron (544 mg k g^{-1}), manganese (49.67 mg k g^{-1}) and boron (21.33 mg k g^{-1}).

Crop husbandry: A fine seedbed was prepared by two dry cultivations and soaking irrigation and at optimum moisture experimental soil was cultivated twice each followed by planking. Chickpea was sown with hand drill by using 65 kg ha^{-1} seed rate. To get optimum plant populations, chickpea plants were thinned out by keeping one plant per hill at 15 days after sowing. Crop was once irrigated at flowering stage to save from moisture stress. Before sowing, recommended dose of N: P: K (32: 84: 60 Kg ha^{-1}), respectively were applied during seedbed preparation. Intercultural operation was carried out immediately after hand weeding at 50 days after sowing. 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 (Figs. 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 seeds per pod, pod weight and 100 seeds weight. Two weeks after every foliar spray of growth regulators, 10 leaves sample were taken from each experimental unit to observe the antioxidant enzyme activities. All the leaves samples were frozen, dried and then 0.5 g powder taken from freeze-dried leaves sampled obtained previously selected tagged plants were homogenized with 50mM Na_2HPO_4-NaH_2PO_4 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 & Maehly, 1955) and superoxide dismutase (SOD) (Giannopolitis & Reis, 1997). While leaf total soluble protein was determined by adopting the procedure and following formula proposed by Bradford (1976).

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

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

$$\text{Harvest index (\%)} = \frac{\text{Seed yield}}{\text{Biological yield}} \text{x 100}$$

Statistical analysis

Data collected during the growth period was statistically analyzed by using the M STAT software and significant differences among various treatment means were competed by using LSD test at 5% probability level (Steel *et al*., 1997).

Results

Plant height (m): Results showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the final plant height during both growing seasons (Table 1). Exogenous application of phytohormones under heat stress conditions significantly improved the plant height of chickpea cultivars during both growing years. However, foliar spray of MLE on both chickpea cultivars DHUST and Bhakkar 2011 cultivated on 15th November produced significantly taller plants during 2017-18 and 2018-19, respectively. Minimum plant height was perceived with the foliar application of tap water on Bhakkar 2011 cultivated on 1st December during both growing seasons (Table 1).

Number of pods per plant: Number of pods per plant contributes extensively to the economic yield. Genetic characters, optimal crop management practices, availability of fundamental nutrients and agro-ecological circumstances are substantially affected the setting of pods. Results showed that interaction among foliar spray of phytohormones 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 phytohormones under heat stress conditions significantly improved the number of pods of both chickpea cultivars. However, foliar spray of MLE on chickpea cultivar Bhakkar 2011 cultivated on 15th November produced maximum pods per plant. While, minimum pods per plant were detected with the foliar application of tap water on chickpea cultivar DUSHT cultivated on 1st December during both growing years (Table 1).

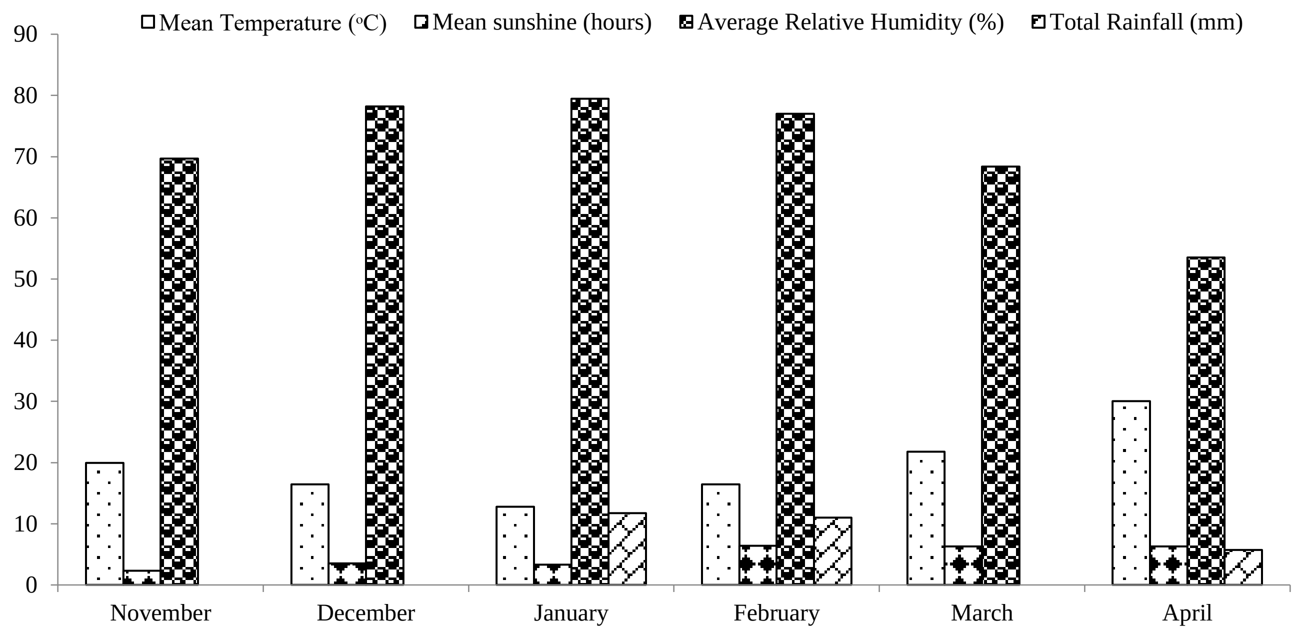

Fig. 1a. Meteorological data for chickpea growth period during 2017-18.

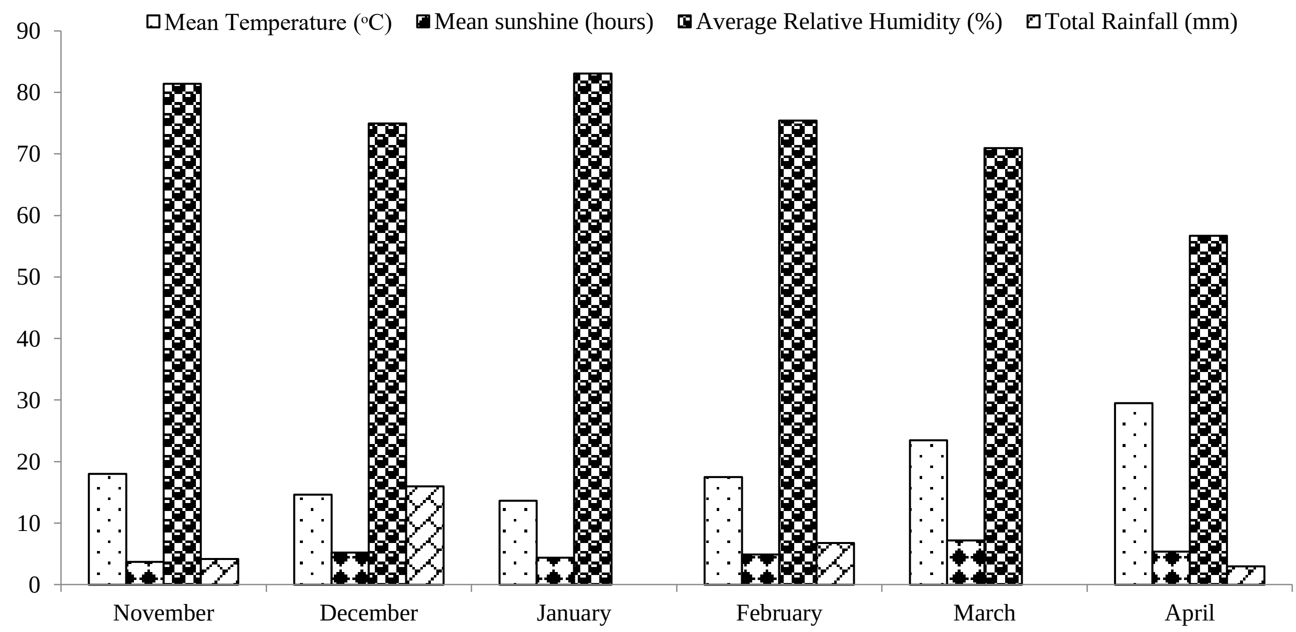

Fig. 1b. Meteorological data for chickpea growth period during 2018-19.

Number of seeds per pod: It is a fundamental feature, which has promising influence on the final productivity. Statistically analysis of the data showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the number of seeds per pod during both growing seasons (Table 1). Exogenous application of MLE on chickpea cultivar Bhakkar 2011 cultivated on 15th November produced maximum seeds per pod during 2017-18 and 2018-19. While, minimum number of seeds per pod was perceived with the foliar application of tap water on chickpea cultivar DHUST cultivated on 1st December during both growing years (Table 1).

Pod weight (g): Statistically analysis of the data showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the pod weight during both growing seasons (Table 1). Exogenous application of MLE on chickpea cultivar Bhakkar 2011 cultivated on 15th November produced heavier pods during 2017-18 and 2018-19. The least pod weight was perceived with the foliar application of tap water on chickpea cultivar DUSHT cultivated on 1st December during both growing years (Table 1).

100 seeds weight (g): Among the different attributes contributing towards economic yield of a crop, hundred-seed weight is of key importance. Statistically analysis of the data showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the number of seeds per pod during both growing years (Table 1). Exogenous application of phytohormones on both chickpea cultivars produced significantly higher 100 seeds weight during both growing seasons. However, foliar spray of MLE on chickpea cultivar Bhakkar 2011 cultivated on 15th November produced significantly higher 100 seeds weight. The foliar spray of tap water on chickpea cultivar DUSHT cultivated on 1st December produced minimum 100 seeds weight.

Biological yield (Kg ha^{-1}): Biological yield is demonstrates the growth and metabolic effectiveness of the crop plants that eventually affects the crop productivity. Results showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the biological yield during both growing seasons (Table 1). Exogenous application of phytohormones under heat stress conditions significantly improved the biological yield of both chickpea cultivars. However, foliar spray of MLE on chickpea cultivar Bhakkar 2011 cultivated on 15th November time produced significantly higher biological yield during 2017-18 and 2018-19. The minimum biological yield was observed with the foliar spray of tap water on chickpea cultivar DUSHT cultivated on 1st December during both growing years (Table 1).

Seed yield (Kg ha^{-1}): Seed yield reveals the collective outcomes of numerous yield components developed under the certain environment. Statistically analysis of the data showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the seed yield during both growing seasons (Table 2). Exogenous application of phytohormones under heat stress conditions significantly improved the seed yield of both chickpea cultivars. However, exogenous application of MLE on chickpea cultivar Bhakkar 2011 cultivated on 15th November produced significantly higher seed yield during both growing seasons. While, minimum seed yield was perceived with the foliar spray of tap water on chickpea cultivar Bhakkar 2011 cultivated on 1st December during both growing years (Table 2).

Harvest index (%): Harvest index represents physiological capacity to mobilize photosynthates towards economic parts. Results showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the harvest index during both growing seasons (Table 2). Foliar spray of MLE on chickpea cultivar Bhakkar 2011 cultivated on 15th November produced maximum harvest index during 2017-18 and 2018-19. While, least harvest index was detected with the foliar application of tap water on chickpea cultivar DHUST cultivated on 1st December during 2017-18 and 2018-19 (Table 2).

Leaf total soluble protein (mg g^{-1}): Results showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the leaf total soluble protein during both growing seasons (Table 2). Foliar spray of MLE on chickpea cultivar Bhakkar 2011 cultivated on 15th November produced significantly higher leaf total soluble protein enzyme during 2017-18 and 2018-19. The foliar spray of tap water on chickpea cultivar DUSHT cultivated on 1st December during 2017-18 and 2018-19 exhibits least leaf total soluble protein (Table 2).

Superoxide dismutase (IU min^{-1}mg^{-1} Protein): Superoxide dismutase determines the first track of defense through detoxification of superoxide radicals; therefore maintain membranes of plant tissue. Results showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the superoxide dismutase enzyme activity during both growing seasons (Table 2). Foliar spray of MLE on chickpea cultivar Bhakkar 2011 cultivated on 1st December produced significantly higher superoxide dismutase during 2017-18 and 2018-19. The least activity of superoxide dismutase was detected with the foliar application of tap water on chickpea cultivar DHUST cultivated on 15th November during both growing years (Table 2).

Peroxidase (POD) (IU min^{-1}mg^{-1} protein): Statistical analysis of the data showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the peroxidase activity during both growing seasons (Table 2). Exogenous application of phytohormones under heat stress conditions significantly improved the peroxidase of both chickpea cultivars. However, exogenous application of MLE on chickpea cultivar Bhakkar 2011 cultivated on 1st December showed significantly higher peroxidase activity. The minimum activity peroxidase was observed with the foliar spray of tap water on chickpea cultivar DHUST cultivated on 15th November during both growing years (Table 2).

Catalase (CAT) (IU min^{-1}mg^{-1} protein): Catalase is an important enzyme observed in almost each living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. Statistical analysis of the data showed that interaction among foliar spray of phytohormones on both chickpea cultivars and their sowing date significantly affected the activity of catalase enzyme during both growing seasons (Table 2). Exogenous application of phytohormones under heat stress conditions significantly improved the catalase enzyme activity of both chickpea cultivars. However, exogenous application of MLE on chickpea cultivar Bhakkar 2011 cultivated on 1st December produced significantly higher catalase. While, minimum activity of catalase was observed with the foliar spray of tap water cultivated on 15th November during 2017-18 and 2018-19, respectively (Table 2).

Table 1. Effect of phytohormones on plant height (m) 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	
		15^{th}Nov.	1^{st} Dec.	15^{th}Nov.	1^{st} Dec.	15^{th}Nov.	1^{st} Dec.	15^{th}Nov.	1^{st} Dec.
Plant height (m)	Tap water	0.583c-f	0.537ef	0.587b-e	0.510f	0.650ab	0.583ab	0.620ab	0.547b
	MLE	0.677a	0.600b-e	0.660ab	0.567d-f	0.693a	0.633ab	0.677a	0.600ab
	BAP	0.657a-c	0.570d-f	0.637a-d	0.540ef	0.670a	0.610ab	0.633ab	0.583ab
	LSD 0.05*p*=	**0.0746**				**0.1110**			
No. of pods per plant	Tap water	24.97cd	20.61d	33.41ab	24.29cd	26.30c-e	22.11e	34.07a-c	28.12c-e
	MLE	27.55bc	25.84cd	38.16a	29.08bc	30.89b-d	27.57c-e	39.82a	33.08a-d
	BAP	26.26cd	24.51cd	36.04a	26.84c	27.99c-e	25.17de	37.37ab	30.17b-e
	LSD 0.05*p*=	**6.2080**				**8.5283**			
No. of seeds per pod	Tap water	1.30d	1.27d	1.59b	1.40cd	1.37e	1.30e	1.62a-c	1.42de
	MLE	1.47bc	1.39cd	1.76a	1.56b	1.56cd	1.42de	1.75a	1.59bc
	BAP	1.40cd	1.40cd	1.75a	1.52bc	1.43de	1.39e	1.73ab	1.55cd
	LSD 0.05*p*=	**0.1468**				**0.1465**			
Pod weight (g)	Tap water	0.243ab	0.233b	0.257ab	0.247ab	0.240de	0.237e	0.253b-d	0.240de
	MLE	0.253ab	0.247ab	0.268a	0.260a	0.250b-e	0.247c-e	0.270a	0.263ab
	BAP	0.250ab	0.243ab	0.267a	0.260a	0.253b-d	0.247c-e	0.260a-c	0.257a-c
	LSD 0.05*p*=	**0.0259**				**0.0161**			
100 seeds weight (g)	Tap water	170.56c-e	160.10e	185.94bc	172.89c-e	167.22cd	158.44d	183.61a-c	170.56cd
	MLE	186.76bc	173.69c-e	204.17a	180.64bd	183.47a-c	170.62cd	201.84a	183.98a-c
	BAP	181.81bd	167.57de	193.40ab	176.01ce	179.48b-d	164.24cd	196.06ab	178.35b-d
	LSD 0.05*p*=	**17.208**				**21.268**			
Biological yield (Kg ha^{-1})	Tap water	4801.0cd	3839.6d	6464.1ab	4708.4cd	5034.3cd	4539.6d	6397.4a-c	5241.7cd
	MLE	5072.5cd	4761.0cd	7419.7a	5720.4bc	5639.1b-d	5194.3cd	7476.4a	6387.1a-c
	BAP	4926.8cd	4689.6cd	7041.1a	5326.6bc	5260.1cd	4856.3cd	7174.5ab	5793.3b-d
	LSD 0.05*p*=	**1262.3**				**1597.7**			

Table 2. Effect of phytohormones on economic yield (kg ha^{-1}), leaf soluble protein (mg g^{-1}) and enzymatic antioxidants (IU min^{-1}mg^{-1} protein) of chickpea.

	Foliar spray	2017-18				2018-19			
		Sowing date of chickpea cultivars				Sowing date of chickpea cultivars			
		DHUST		Bhakkar-2011		DHUST		Bhakkar-2011	
		15^{th}Nov.	1^{st} Dec.	15^{th}Nov.	1^{st} Dec.	15^{th}Nov.	1^{st} Dec.	15^{th}Nov.	1^{st} Dec.
Seed yield (Kg ha^{-1})	Tap water	1396.0de	1091.8e	1928.7bc	1370.1de	1469.4de	1213.3e	1919.5a-d	1503.0c-e
	MLE	1596.8cd	1474.3de	2381.8a	1734.7cd	1800.0b-d	1595.5c-e	2470.7a	2034.9a-c
	BAP	1497.8c-e	1377.2de	2180.3ab	1607.7cd	1602.7c-e	1414.6de	2244.8ab	1752.5b-e
	LSD 0.05*p*=	**441.94**				**555.42**			
Harvest index (%)	Tap water	29.05cd	28.35d	29.89b-d	29.04cd	29.18cd	26.66e	29.95b-d	28.66de
	MLE	31.50ab	30.86a-c	32.03a	30.34a-d	31.87ab	30.53b-d	33.02a	31.94ab
	BAP	30.43a-c	29.26cd	30.86a-c	30.10a-d	30.53b-d	29.01cd	31.06a-c	30.23b-d
	LSD 0.05*p*=	**2.0755**				**2.3329**			
Leaf total soluble protein (mg g^{-1})	Tap water	0.723ab	0.640b	0.740ab	0.687ab	0.667bc	0.620c	0.740a-c	0.673bc
	MLE	0.763ab	0.707ab	0.790a	0.697ab	0.737a-c	0.693a-c	0.813a	0.723a-c
	BAP	0.747ab	0.697ab	0.770a	0.707ab	0.707a-c	0.657c	0.787ab	0.693a-c
	LSD 0.05*p*=	**0.1244**				**0.1239**			
Superoxide dismutase (IU min^{-1}mg^{-1} protein)	Tap water	43.71f	126.66d	50.96ef	139.91cd	39.77e	130.45c	48.59de	136.56bc
	MLE	59.30ef	152.30bc	64.25e	173.78a	52.53de	147.79a-c	59.16d	160.44a
	BAP	55.20ef	145.34c	59.57ef	164.75ab	47.00de	139.01bc	53.21de	152.74ab
	LSD 0.05*p*=	**17.723**				**18.459**			
Peroxidase (IU min^{-1}mg^{-1} protein)	Tap water	6.85d	12.52c	7.80d	15.45b	6.52e	13.19d	7.60e	15.12cd
	MLE	8.23d	18.83a	9.08d	20.41a	7.95e	17.93ab	8.15e	19.94a
	BAP	7.93d	15.84b	8.57d	19.27a	7.67e	16.07bc	7.95e	18.47ab
	LSD 0.05*p*=	**2.2663**				**2.5059**			
Catalase (IU min^{-1}mg^{-1} protein)	Tap water	24.04g	63.91d	28.79fg	74.74c	23.84g	60.58d	28.82fd	73.07c
	MLE	33.06ef	80.94bc	39.07e	94.77a	29.73fg	79.27bc	41.40e	91.43a
	BAP	31.73e-g	77.06c	37.39ef	88.86ab	26.77fg	74.40c	35.89ef	85.53ab
	LSD 0.05*p*=	**8.8164**				**9.3126**			

Discussion

Heat stress causes irrecoverable damage to plant growth, thus adversely affecting the plant growth and economic yield. One of the ways to mitigate the negative influences of heat stress may involve determining certain natural or synthetic molecules that have the potential to protect the plants from the harmful influences of heat stress (Shafiq *et al*., 2021). Previous results showed that phytohormones such as Benzyl-amino purine (BAP) and moringa leaf extract (MLE) improved the plant growth and productivity under various abiotic stresses (Yasmeen *et al*., 2013). Hence, current investigation was planned to use phytohormones to mitigate the harmful effects of heat stress and improved the chickpea growth and yield. Results of present study showed that foliar application of moringa leaf extract on chickpea significantly improved the plant height. As it works like a potential plant growth promoter having a balance of micro and macronutrients, zeatin, carotenoids, ascorbates, antioxidants and phenols. Therefore, it is suggested that exogenously applied MLE could have potential to enhance the endogenous hormonal levels of plants thus resulting in higher plant growth even under heat stress circumstances (Shafiq *et al*., 2021).

Heat stress during the reproductive stage considerably influenced numerous yield-contributing attributes (Snider *et al*., 2010). Results of our study showed that terminal heat stress adversely influenced the yield contributing attributes such as pods per plant, pod weight, seeds per pod, 100-pods weight that eventually reduced the overall biomass and seed yield. This could be due to the fact that terminal heat stress might interfere with the process of pollination and fertilization leading in reduction of fruit set (Yasmeen & Muzamil, 2022). However, foliar spray of phytohormones under such situations mitigates these negative influences of heat stress and enhance the productivity. Though both the phytohormones mitigated the negative influences of heat stress and enhanced the productivity, but foliar spray of MLE results were superior. It might be due to the fact that MLE mitigate the negative influences of terminal heat stress on late sown chickpea cultivars (Yasmeen & Muzamil, 2022). Similarly, it was also observed that MLE enhanced the photosynthesis rate and transport of photo-assimilates toward pods to improve the carbohydrate delivery during pods development phase even under stress conditions (Blaise *et al*., 2009). It was also observed that foliar spray of MLE enhanced the enzymatic antioxidants activities in both chickpea cultivars under heat stress circumstances. Previous results also confirmed that exogenous application of MLE on wheat under abiotic stress conditions also enhanced the antioxidant activities (Yasmeen *et al*., 2013). Moreover, Hussain *et al*., (2020) established that foliar spray of MLE on cotton plants cultivated under heat stress conditions increase the production of endogenous antioxidants. This initiation of self-protection system might be related with higher contents of nutrients element (K^+ and Ca^{2+}) along with growth enhancers (zeatin and gibberellins) existed in moringa leaves because mineral elements are activator and cofactor of various antioxidant enzymes (Arif *et al*., 2019).

Conclusion

Results of present study suggested that foliar spray of phytohormones particularly MLE modulated the heat stress induced losses to chickpea plants by improving their antioxidant defense mechanism and enhanced the productivity of chickpea crop.

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