ACCEPTED ARTICLE

Potassium foliar application to enhance high temperature tolerance and productivity of canola under late sown conditions

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Abstract

Potassium (K) mitigates the effect of high temperature on canola, especially during its later growth stages when sowing of canola is delayed. To explore the effect of K on high temperature tolerance and canola production a field experiment [2019 – 2021] was conducted. The experiment had two sowing dates (October 15 and November 1) and four K treatments, i.e. control, soil application (50 kg K ha⁻¹), soil application + 0.25% K foliar spray at pre- and post-flowering stages and soil application + 0.50% K foliar spray at pre and post-flowering stages. Application of 50 kg K ha⁻¹ as soil application along with 0.50% K-foliar sprays resulted in higher production of biochemicals (superoxide dismutase, peroxidase, catalase, and total soluble sugars) besides net photosynthetic rate and stomatal conductance along with less malondialdehyde production and relative cell injury in crops sown on November 1st than untreated plants. Moreover it also enhanced chlorophyll florescence and chlorophyll (a and b) contents of late- sown crops. Plants sown on November 1st and received 50 kg K ha⁻¹ as soil application along with 0.50% K foliar spray also gave higher yield components, yield and economic returns than control. Therefore, it is suggested to supply 50 kg K ha⁻¹ at sowing and a foliar spray of 0.50% K at the pre- and post-flowering stages to canola crops sown late in the season to achieve optimal and economical yield levels.

27 to 0

Keywords: high temperature; reactive oxygen species; antioxidant activities; foliar spray; lipid per oxidation.

INTRODUCTION

Climate change is contributing to global warming, which has implications for the regional distribution and cultivation conditions of crops (Nesar et al., 2022). From last many years, the air temperature of earth is increasing steadily and it is expected that the rise in temperature will continue which would result in significant rise in average temperature of earth (IPCC, 2018). High

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temperature can have a severe impact on crop productivity (Mostofa et al., 2022). Canola is vulnerable to high temperature especially during its reproductive stage (Annisa et al., 2013). In canola the highly heat sensitive stage is flowering stage and considerable yield reduction occurs when temperature goes higher than 28-30°C at flowering stage (Chen et al., 2020). High temperature can cause abnormal flower development, leading to fewer pods and seeds (Chen et al., 2021). The problem is further exacerbated when canola planting is delayed due to intensive cultivation practices. Farmers often struggle to plant canola on time, especially after cultivating kharif crops like cotton, rice, and potatoes (Yousaf et al., 2002). Pakistan is among the countries which are facing the severity of climate change. Based on records from the International Disasters Database (EM-DAT), Pakistan has experienced a significant rise in the frequency and intensity of extreme meteorological as well as hydrological events, including droughts, storms, floods and extreme temperatures, over the past three decades. In addition to that the annual average temperature of Pakistan has also increased up to 1.68°C during 1901 to 2021 whereas the global rise of average annual temperature during the same period is 1.1°C; hence the increase in Pakistan's average annual temperature is also higher than the global annual average temperature rise. Fertilization strategies significantly reduce abiotic stress impacts by promoting plant development, structure, biochemical processes, and nutrients stores, enabling plants to tolerate nostile ecological circumstances (Xu et al., 2021). Within vital group of macronutrients, K assumes a predominantly pivotal part in affecting growth and development of plant, whether in typical or challenging environmental conditions. Its primary function in plants revolves around boosting stress tolerance. Through its ability to decrease transpiration rates and enhance water absorption, K contributes to increased agricultural productivity (Aslam et al., 2021). Furthermore, it assists in preserving turgidity of cell and counteracting detrimental impacts of reactive oxygen species (ROS) (Jan et al., 2019). Enzymatic activities and metabolic processes are enhanced by K (Zaman et al., 2019), thus enhancing physiological systems and building up antioxidant defense mechanisms (Hasanuzzaman et al., 2020). Importantly, K performs a pivotal function in stress mitigation by accelerating the metabolism of plant proteins, which regulate numerous plant processes during adverse environmental conditions. Furthermore, it supports proline synthesis, contributes to osmotic regulation and bolsters plant resilience to stress (Zamani et al., 2020). Implementing proper K fertilization strategies can, therefore, perform a crucial part in boosting productivity and health of plant, even in harsh conditions. Li et al. (2023) found that the application of potassium dihydrogen phosphate foliar spray at flag leaf stage in wheat resulted in increase in net photosynthetic rate (Pn), chlorophyll content, antioxidant enzymes activity, dry matter accumulation, yield related traits and overall yield of crop as well as quality of produce facing high temperature. Likewise, Sarwar *et al.* (2022) reported that high temperature considerably damaged physiology of leaf as well as grain yield of wheat. Nonetheless, existing data are scarce concerning the effectiveness of K foliar spray for mitigation of high temperature effects in canola plants. Consequently, current research project was formulated with the following aims: (i) to monitor the physiological and developmental responses of canola under high temperature conditions; (ii) to appraise the influence of K foliar spray on physiology of canola which enables the plant to cope with high temperature; and (iii) to evaluate the influence of K foliar spray on canola yield.

MATERIAL AND METHODS

Experimental Site

Experiment was carried out at the Agronomic Research Station Khanewal, Pakistan (Fig. 1). This field trial was conducted for two growing seasons, from 2019 to 2021. The experimental soil was sandy loam with an 8.6 pH, 4 ds cm⁻¹ electrical conductivity (EC), 0.06% nitrogen (N), 6.9 ppm phosphorus (P), and 206.7 ppm K.

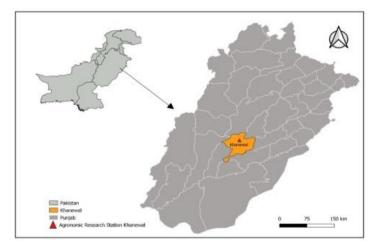


Fig. 1: Geographic location of the experimental site.

Experimental Treatments and Design

The experiment was laid out in RCBD with split-plot arrangement with two sowing dates (main plots) and four K application treatments (sub-plots) having net plot size on $7.0 \text{ m} \times 2.7 \text{ m}$ and three replications. Sowing dates were: October 15th; November 1st and K treatments were: T_1 : control

(no application of K), T₂: 50 kg K ha⁻¹ (soil applied), T₃: T₂ + foliar spray of 0.25% K at pre- and post-flowering stages, T₄: T₂ + foliar spray of 0.50% K at pre- and post-flowering stages. The canola variety Faisal Canola was used in the study. Potassium nitrate (KNO₃) was used as K source. and K solutions were prepared distilled water as solvent and sprayed manually using a hand sprayer.

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Crop husbandry

Canola was planted in rows 45 cm apart with a rabi drill using 4 kg ha⁻¹ seed rate. At four-leaf stage, the crop was thinned to maintain 10 cm plant to plant distance. Weeds were removed manually when required. Overall 85 kg N and 74 kg P ha⁻¹ were given to crop; with all P and K fertilizers applied at sowing time and N fertilizer was applied in two equivalent splits at sowing and flowering. Three irrigations were provided, at 30 days after sowing, flowering, and pod formation. Harvesting for treatments sown on October 15th and November 1st took place on March 8th and March 24th, respectively, in both years of the study.

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Observations

Leaf Biochemical Analysis

Leaf samples (from middle portion of main branch) were collected 10 days after K-foliar sprays application to recorded superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), total soluble sugars (TSS) and malondialdehyde (MDA). The SOD (U/mg protein) activity was determined using procedure described by Winterbourun et al. (1993). The (POD) (U/mg protein) activity was determined by employing the procedure described by Ogawa et al. (1985). The amount of CAT (U/mg protein) was determined by following the procedure described by Sinha (1972). The TSS (mg/g dry weight) was determined using the procedure described by Dubois et al. (1956). The MDA (U/mg) was determined by employing the procedure described by Velikova et al. (2000).

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Relative Cell Injury

Ten days following the K-foliar spray treatment, fresh leaf samples (from the middle region of the main branch) were taken to record relative cell injury. The following formula was used (Lutts et al., 1999). Electrical conductivity (EC) was measured using EC meter (Model, Jenway 4510, Japan).

120 Relative Cell Injury (RCI %)= (EC of the sample - EC of the control) / (EC of the maximum leakage) × 100

Photosynthetic Parameters

Ten days following the application of K-foliar sprays, the net photosynthetic rate (Pn, μmol m⁻² s⁻¹) and stomatal conductance (Gs, mol m⁻² s⁻¹) were measured using infrared gas analyzer analyzer with Broad Head, Part Number LCi-002/B with Serial Number 32455 (Sarwar et al., 2022). Chlorophyll fluorescence (Fv/Fm) was recorded to measure thylakoid membrane stability. Chlorophyll was extracted from leaf samples (from middle portion of main branch) using a standard procedure and the fluorescence was measured using a chlorophyll fluorimeter (OptiScience, UK with Serial Number 0729501) (Murchie and Lawson, 2013). Fresh leaf samples (from middle portion of main branch) were collected 10 days after K-foliar sprays to record chlorophyll a/b using the following equations (Lichtenthaler and Wellburn, 1983):

Chlorophyll a = 12.7 (A663) - 2.69 (A647)

Chlorophyll b = 22.9 (A647) - 4.68 (A663)

Yield and Yield Related Traits

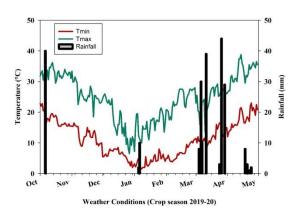
When the plants reached maturity, ten were chosen at random from each plot. Measurements included plant height, number of branches and pods per plant, and length of pod. The quantity of seeds per pod was calculated by counting and threshing fifteen pods that were taken from the chosen plants. After that, the complete plots were gathered and threshed. A total of 1000 seeds were weighed and counted in order to record the weight. The yield per plot was calculated by taking the total weight of seeds in each plot and converting it to yield per hectare.

Economic Analysis

The gross income per hectare (Rs.) was calculated by multiplying canola seed yield (kg ha⁻¹) by the market rate (Rs. /kg) of canola seed (Byerlee, 1988). Fixed and variable costs per hectare (Rs. /ha) were calculated by combining the costs associated with standard field operations and treatment-specific expenses, respectively. The total cost of production (Rs. /ha) was obtained by combining the fixed and variable costs. Net income (Rs. /ha) was calculated by deducting the total cost of production from the gross income. The benefit-cost ratio (BCR) for each treatment was determined by dividing the net income by the total cost of production.

Weather

During 2019-20, average maximum temperature was 24.50°C, while average minimum temperature was 11.36°C and total rainfall was 232 mm from October to April. However, from February to March of 2020, when the crop was in the reproductive stage, the highest temperature range was 25.50–36.50°C. During 2020-21, average maximum temperature was 27.27°C, while average minimum temperature was 10.87°C and total rainfall was 64.20 mm from October to April. However, in February and March of that year the highest temperature range was 22.50–35.50°C.



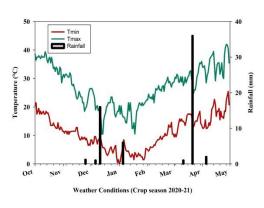
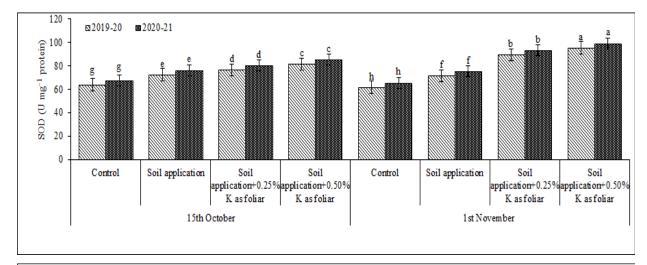


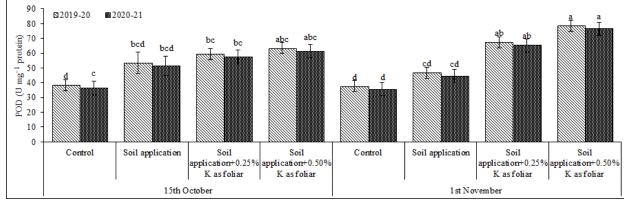
Fig. 2: Climate conditions at the experimental location throughout the study duration.

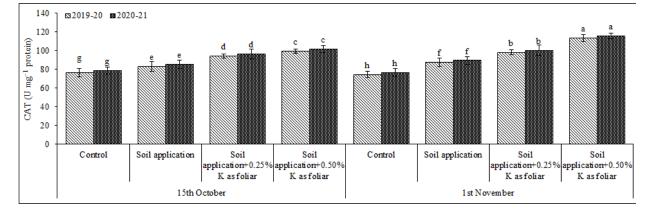
RESULTS Leaf Physiology

Late sowing reduced SOD, POD, CAT, and TSS contents in leaves, and increased MDA production. Foliar sprays of K enhanced these contents and reduced MDA production, especially for plants sown on November 1st (Fig. 3, 4). Late sowing resulted in high RCI, which was reduced by foliar sprays of K, especially at 0.50% concentration (Fig. 4). Delayed sowing reduced Pn and Gs, which were preserved by foliar spray of K, especially at 0.50% concentration (Fig. 4). Late sowing also reduced chlorophyll fluorescence and leaf chlorophyll contents (a and b), which were maintained by foliar sprays of K, especially at 0.50% concentration (Fig. 5). Overall, foliar sprays of K mitigated the negative effects of late sowing on canola plants.

Potassium mitigates effects of high temperature in canola







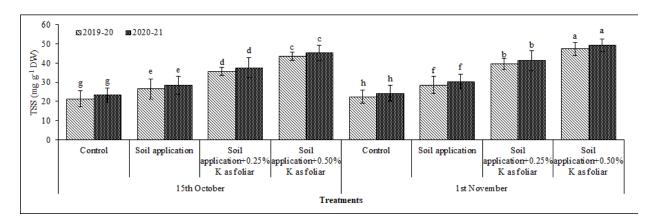
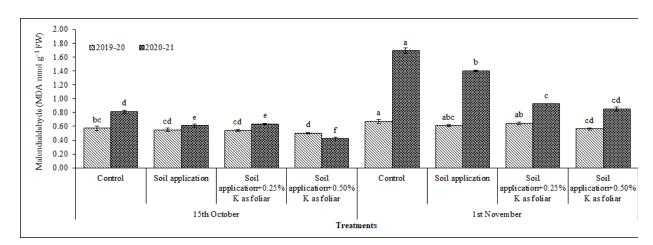
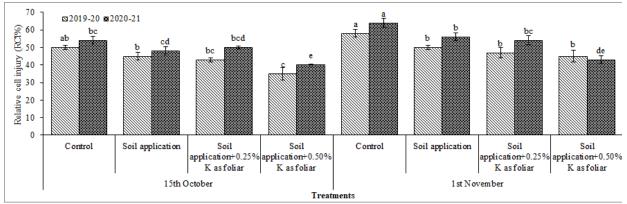


Fig. 3: Influence of two sowing dates and potassium application on biochemical of canola. Means sharing same case letter do not differ significantly at P<0.05.





Potassium mitigates effects of high temperature in canola

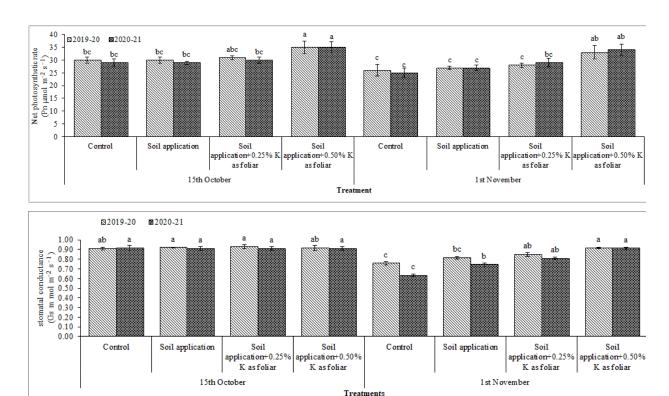
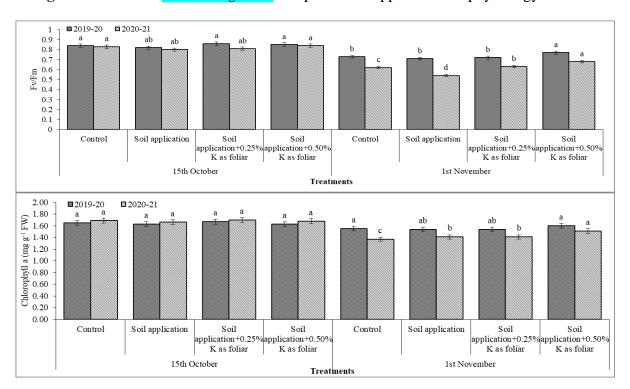


Fig. 4: Influence of two sowing dates and potassium application on physiology of canola.



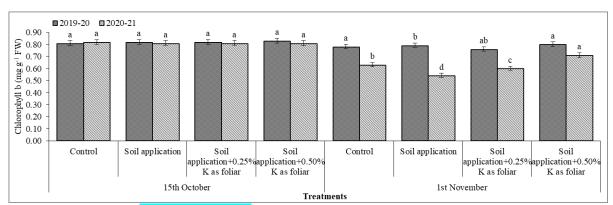


Fig. 5: Influence of two sowing dates and potassium application on chlorophyll fluorescence (Fv/Fm) and chlorophyll contents of canola.

Yield and Yield Related Traits

Data regarding yield and related traits has been presented in table 1. During 2019-20, the canola crop sown on 15th of October resulted in 9% higher plant height as compared to canola crop sown on November 1st. Moreover regarding number of pods per plant, 52% higher were recorded in crop sown on October 15th and sprayed with 0.50% K-foliar spray than crop sown on same date but no K-foliar spray was applied. Additionally, regarding pod length and number of seed per pod, 10% and 21% higher pod length and number of seed per pod, respectively, were documented in plants sprayed with 0.50% K-foliar spray as compared with control. Regarding 1000-seed weight, 14% higher was recorded when canola was sown on October 15th than canola sown on November 1st, whereas among K treatments, the 0.25 and 0.50% K-foliar sprays resulted in 29 higher 1000-seed weight than control. Regarding yield, canola crop sown on October 15th and sprayed with 0.50% K-foliar spray resulted in 7% higher yield as compared with crop sown on same date but not sprayed with K. Whereas, crop sown on November 1st and sprayed with 0.50% K-foliar spray resulted in 43% higher yield as compared with crop sown on similar date but not sprayed with K at pre- and post-flowering stages.

During 2020-21, the canola crop sown on 15th of October resulted in 4% and 17% higher plant height and number of branches per plant, respectively, than canola crop sown on November 1st. Moreover regarding number of pods per plant, 39% higher were recorded in crop sown on November 1st and sprayed with 0.50% K-foliar spray than crop sown on same date but no K foliar spray was applied. Additionally, regarding pod length, 16% higher pod length was documented in plants sown on October 15th than plants sown on November 1st whereas among K treatments, crop sprayed with 0.50% K-foliar sprays gave 7% higher pod length as compared with control.

Furthermore, regarding number of seed per pod, 48% higher number of seed per pod was recorded in plants sown on October 15th as compared to plants sown on November 1st whereas among K treatments, crop sprayed with 0.50% K-foliar sprays gave 48% higher number of seed per pod as compared with control. Regarding 1000-seed weight, 14% higher was recorded when canola was sown on October 15th than canola sown on November 1st, whereas among K treatments, the 27% higher 1000-seed weight as compared with control. In case of yield, canola crop sown on October 15th and sprayed with 0.50% K-foliar spray resulted in 7%, higher yield as compared with crop sown on same date but not sprayed with K. Whereas, crop sown on November 1st and sprayed with 0.50% K-foliar spray resulted in 43% higher yield as compared with crop sown on similar date but not sprayed with K at pre- and post-flowering stages.

Table 1: Influence of two sowing dates and potassium application on yield and related traits of canola.

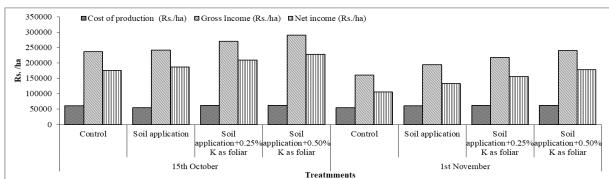
ϵ	er of	Mirror										
nla	plant		Number of pods per Plant		(cm)		Number of seeds per pod		1000-seed weight (g)		vield (g)	
2019- 20 2020-21 2019- 20 20	020-21	2019- 20	2020-21	2019- 20	2020- 21	2019- 20	2020- 21	2019- 20	2020- 21	2019- 20	2020-	
Sowing dates (D)												
15th October (D ₁) 197A A 6	6 A	374 A	238 A	6.80	7.43 A	17	23 A	4.14 A	4.32 A	1170 A	2606 A	
1st November (D ₂) 179 B 163 B 5	5 B	319 B	171 B	6.60	6.22 B	17	18 B	3.58 B	3.76 B	723 B	2232 B	
HSD 10.79 4.19 -	0.16	10.79	17.14	-	0.34	-	1.26	1.06	1.24	43.56	32.66	
Treatments (T)												
Control (T ₁) 182 165 5	5	254 D	175 C	6.43 B	6.54 B	15 C	13 C	3.04 C	3.22 C	849 D	2037 D	
50 kg K ha ⁻¹ (T ₂) 193 165 5	5	334 C	198 BC	6.63 B	6.68 AB	17 B	21 B	3.90 B	4.08 B	890 C	2278 C	
0.25% K+50 kg K ha ⁻¹ 190 168 5 (T ₃)	5	379 B	209 AB	6.58 B	6.99 A	17 B	23 A	4.29 A	4.38 A	951 B	2601 B	
0.50% K+50 kg K ha ⁻¹ 190 168 5	6	419 A	235 A	7.17 A	7.07 A	19 A	25 A	4.20 A	4.47 A	1094 A	2760 A	
HSD	-	26.86	31.37	0.52	0.44	1.52	1.78	0.18	0.18	41.23	87.11	
		D	×T									
D_1T_1 167 170 5	6	234 d	219 ab	6.56	7.17	14	15	3.35	3.53	1156 bc	2280 de	
D ₁ T ₂ 188 169 6	5	339 с	237 a	6.63	7.32	18	24	4.12	4.30	1097 c	2413 cd	
D ₁ T ₃ 181 171 6	6	436 b	243 a	6.60	7.60	18	27	4.59	4.77	1186 ab	2752 b	
D ₁ T ₄ 180 171 6	6	487 a	253 a	7.43	7.61	20	26	4.49	4.67	1240a	2980 a	
D ₂ T ₁ 196 160 5	5	273 d	132 c	6.30	5.91	16	12	2.72	2.90	543 f	1794 f	

D_2T_2	197	160	5	5	328 c	160 c	6.63	6.04	16	18	3.69	3.87	684 e	2143 e
D_2T_3	198	165	4	5	322 c	174b c	6.57	6.53	16	22	3.99	4.17	715 e	2450 с
D ₂ T ₄	199	165	5	5	351 c	216 ab	6.90	6.37	18	20	3.92	4.10	949 d	2540 c
HSD	-	-		-	46.3 0	53.82	-	-	-	-		-	31.2 5	150.1 5

Means sharing same case letter do not differ significantly at P<0.05

Economic Analysis

When the crop was sowed on October 15th and treated with 0.50% K-foliar sprays at the pre- and post-flowering stages, compared to the control, there was a 23% increase in net revenue and benefit cost ratio according to the economic analysis (Fig. 6). Similarly, when crop was sowed on November 1st and treated with 0.50% K-foliar sprays at pre- and post-flowering stages, compared to control, 40% higher net income was obtained, likewise, when crop was sowed on November 1st and treated with 0.50% K-foliar sprays at pre- and post-flowering stages, compared to control, 32% higher benefit cost ratio was obtained (Fig. 6).



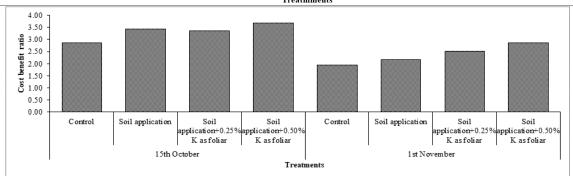


Fig. 6: Economic analysis of two sowing dates and foliar spray treatments of canola.

DISCUSSION

The findings of study demonstrated that K-foliar sprays resulted in improved physiological response and yield of canola plants which are likely to face high temperature at flowering under

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late sowing conditions. Under higher temperature exposure, the plant metabolism as well as many biochemical reactions is disrupted (Hasanuzzaman et al., 2013). Potassium enhances tissue water potentiality, which aids in extreme temperature tolerance, and it helps to activate numerous physiological and metabolic processes like photosynthesis, respiration, and nutrition homeostasis (Hasanuzzaman et al., 2018). In present study the activity of enzymatic antioxidants (SOD, POD, and CAT) was significantly improved by spraying K solution of canola plants sown on 1st November (Fig.3). These enzymes are protein in nature and their function is the transformation of ROS into the form which is non-toxic/harmful for plants, hence play crucial role in defense mechanism of plant which enables plant to mitigate the harmful effect of oxidative stress caused by many abiotic stresses (Saez and Estan-Capell, 2017). These ROS cause lipid peroxidation of the cell membrane (Fang et al., 2022). A higher level of enzymatic antioxidant activity in plants supplied with K-foliar sprays might be due to the role of K in enhancing the production/activity of enzymatic antioxidants in stressed plants (Fang et al., 2022). One way K enhances their production/activity is by the regulation of enzymes activity present in antioxidant defense pathways (Anschütz et al., 2014). Potassium also aids in maintaining cellular ion balance and osmotic potential, which are crucial for enzyme activity and overall plant health under stress. By regulating osmotic balance, K helps prevent cellular dehydration and maintains turgor pressure, which is essential for optimal enzyme function (Siddiqui et al., 2021). In addition, fewer contents of MDA and less occurrence of RCI (Fig. 4) in plants sprayed with K-foliar sprays were also recorded in this study. Malondialdehyde contents in plant leaves are used to estimate the lipid peroxidation (Houmani et al., 2022). As discussed above the K application increase the efficacy and contents of enzymatic antioxidants defense systems in plants (Siddiqui et al., 2021) thus reduce the cellular damage in plants. Hence less content of MDA and less prevalence of RCI might be due to this protective role of K against ROS. Additionally higher level of TSS in plants (Fig. 3) sprayed with K was also recorded. Accumulation of TSS is mainly observed in mild stress which retard the growth of plant but any how the process of photosynthesis is not much inhibited (Keunen et al., 2013). Mainly the production of TSS is taken place during osmotic stress and act as osmo-protectants to stabilize the activity of cell membrane as well as to main the turgor of cell (Dien et al., 2019). Many researchers reported that K enhances the accumulation and assembly of TSS in plants facing abiotic stress through several mechanisms such as osmotic adjustment (Tränkner et al., 2018;), the stimulation

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of numerous enzymes, like RuBisCO (Weng et al., 2007) and governing the functioning of 254 photosynthesis machinery (Tavakol et al., 2022). Translocation of photo-assimilates from source 255 256 to sink is also governed by K (Cakmak, 2005). Net photosynthesis and stomatal conductance (Fig. 4) was also enhanced by the foliar application 257 K on canola, Stomatal opening and closing is governed by K ions (Shabala, 2003). Potassium ions 258 are actively pumped into guard cells causing them to swell and leading to stomatal opening (Lu et 259 al., 2019). This process is crucial for regulating loss of water by transpiration and facilitating the 260 uptake of CO₂ for photosynthesis (Lu et al., 2019). Moreover K also influence the photosynthesis 261 as it performs crucial part in the activation of enzymes vital for photosynthesis, such as Rubisco, 262 which catalyzes the fixation of CO₂ during the Calvin cycle (Rawat et al., 2022). Canola plants 263 sown on November 1st and subjected to 0.50% K-foliar sprays exhibited an augmentation in 264 chlorophyll levels compared to untreated plants (Fig. 5) sown on the same date. Potassium plays a 265 crucial part in the production of precursor molecules for chlorophyll pigments and enhances the 266 capacity to transform radiation energy into chemical energy within the chloroplasts (Zhao et al., 267 268 2001). The supplementation of external K caused a noticeable increase in several important parameters 269 of canola crop sown on November 1st. These parameters included the pod count per plant, seeds 270 per pod, and the weight of 1000 seeds (Table 1). This enhancement in pod and seed quantities can 271 272 likely be attributed to the ability of K to improve growth by its participation in numerous biological 273 functions, such as enzyme activation, assimilate and nitrate transport, water relations, stomatal 274 regulation and photosynthesis (Oosterhuis et al., 2014). The improvement in 1000-seed weight (Table 1) due to the application of K, might be the function of K in photosynthesis and 275 transportation of sugars from source to sink (Oosterhuis et al., 2014). The increase in overall yield 276 (Table 1) observed in canola sown on November 1st, attributed to K application, can be attributed

of these substances to developing seeds (Sarwar et al., 2022). These combined effects contribute 280

to an overall increase in seed yield and benefit cost ratio.

CONCLUSIONS

This study has demonstrated that the foliar application of K triggers antioxidant activity within plants, as evidenced by increased production of SOD, POD, CAT, and TSS. This timely activation

to a combination of factors. These factors include an improvement in membrane stability, enhanced

efficiency of photosynthesis, increased accumulation of carbohydrates, and effective translocation

- of the antioxidant defense system, brought about by K-foliar spraying, led to a significant decrease
- in MDA levels, reduced cell injury, and the maintenance of optimal photosynthesis rates, stomatal
- conductance, and chlorophyll a/b contents. Furthermore, when a combination of 50 kg K ha⁻¹
- applied at sowing and foliar spraying with 0.50% K at both pre- and post-flowering stages was
- employed, higher crop yield and improved benefit-cost ratios were obtained. Based on these
- findings, it is recommended to apply 50 kg K ha⁻¹ at sowing and a foliar spray of 0.50% K at the
- 292 pre and post-flowering stages to canola crop sown late in the season to achieve optimal and
- economical yield levels.

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