

Foliar Benzoic Acid Triggered Agronomic, Physiological and Fiber Quality Traits of Cotton (*Gossypium hirsutum* L.) Under Heat Stress

Running Title: Foliar spray of benzoic acid on heat stressed cotton

Abdul Shakoor^{1*}, Misbah Zulfqar², Ijaz Ahmad³, Ali Sher⁴, Muhammad Zafar⁵, Muhammad Zeeshan⁶, Waheed Arshad⁶, Muhammad Arslan⁷, Muhammad Rizwan Khurshid⁸, Saima Naseer⁹, Saba Saeed⁹, Mustazhar Billah Zafar¹⁰

¹Wheat Program, Crop Sciences Institute, National Agricultural Research Centre, Islamabad Pakistan

²Ayub Agricultural Research Institute Faisalabad Pakistan

³Pulses program, Crop Sciences Institute, National Agricultural Research Centre, Islamabad Pakistan

⁴Cotton Research Sub Station Jhang Pakistan

⁵Sugarcane Research Institute, Ayub Agricultural Research Institute Faisalabad Pakistan

⁶Barani Agricultural Research Station, Fateh Jhang Pakistan

⁷Barani Agricultural Research Institute, Chakwal Pakistan

⁸Agronomic Research Institute, Ayub Agricultural Research Institute Faisalabad Pakistan

⁹Plant Pathological Research Institute, Ayub Agricultural Research Institute Faisalabad Pakistan

¹⁰Applied Linguistics GC University, Faisalabad Pakistan

Abstract

Heat stress at reproductive stages of cotton cause adverse effects on agronomic, physiological attributes and worsens the fiber quality of cotton. A field study was conducted to see the foliar spray of benzoic acid (BZA) could help to alleviate the negative effects of heat stress on agronomic, physiological quality traits of cotton. Two levels of heat treatment were assigned in the main plots, that was, H₀ = no heat stress; H₁ = heat stress imposed at squaring for the period of 7 days. Six levels of foliar spray of benzoic acid i.e. control, water spray, 0.25, 0.50, 0.75 and 1.00 mM were applied in each subplot during heat stress imposition. The crop was sown on 75 cm apart ridges and plant to plant distance was maintained at 30 cm. All other agronomic practices were kept normal and uniform. The standard procedures were adopted for recording the data on various cotton parameters. A remarkable decrease in agronomic, physiological and quality attributes was observed under heat stress compared to control. Foliar application of benzoic acid significantly improved agronomic, physiological and fiber quality. Conclusively, heat stress was more detrimental at squaring for one week than no heat stress for cotton crop in terms of the studied traits. Foliar BZA 0.75 mM is, therefore, recommended to mediate heat stress, due to its beneficial effect on agronomic, physiological and fiber related parameters of cotton.

Key words: Cotton; fiber quality; Cell membrane thermostability; Chlorophyll; high temperature

1. Introduction:

In today's world, the most evident evidence of climate change is high temperatures. The rate of temperature increase in the recent decade (2000-2010) was 2.2% more than the rate of temperature increase in the previous 30 years (1970-2000) (IPCC, 2014). Furthermore,

a 1.5°C increase in earth surface temperature has been seen during the last decade (IPCC, 2018). Cotton enjoys temperature, but it is extremely sensitive to high temperature stress during floral development, which severely limits crop development and yield (Oosterhuis and Snider, 2011). Cotton is cultivated on 33.4 million hectares worldwide, with an average yield of 121.4 million bales (USDA, 2018). Cotton is grown on 2144 thousand hectares in Pakistan, yielding 4.910 million bales per year. It accounts for 1.4% of agricultural value addition and 0.3% of GDP (Government of Pakistan, 2023).

Temperature increases above a certain threshold have a negative impact on plant growth (Saleem *et al.*, 2020). Heat stress enhances the onset of various cotton phenological stages (Ahmad *et al.*, 2017). High temperatures destabilise the photosystem, break down chlorophyll, disrupt enzyme activity, and accelerate lipid peroxidation (Szymańska *et al.*, 2017). All of these physiological disturbances at the end of light reactions culminate in a downregulation of reductant synthesis (NADP⁺). Diminished reducing power synthesis decreases glucose partitioning to fibre production in cotton and other reproductive sections of agricultural plants (Demmig-Adams *et al.*, 2018). Increased temperatures may cause the formation of changed fibre properties such as higher micronaire value, greater fibre strength, and increased fibre maturity (Tonne, 2011). The drop in carbohydrate availability and cellulose production under stress conditions is critical for good fibre development, fibre weight on a seed, and, ultimately, yield loss. Fibre quality is determined by various features such as fibre length, fibre strength, and fibre micronaire (fineness), all of which have varying degrees of sensitivity to environmental variables, particularly high temperatures (Pettigrew, 2008). The temperature range of 25 to 30°C is favourable for cellulose synthesis, while temperatures below or beyond this range result in decreased cellulose production (Roberts *et al.*, 1992).

Synchronization in the source-sink connection may improve fibre quality and seed cotton yield, which are hampered by heat stress (Saleem *et al.*, 2018a, 2018b). Heat stress can be managed in a variety of ways, including adjusting the sowing date (Sarwar *et al.*, 2019), conserving tillage (Khan *et al.*, 2017), reducing the use of synthetic fertilizers (Shahid *et al.*, 2015), and applying growth substances (Kamal *et al.*, 2017a) and nutrients (Saleem *et al.*, 2018c).

Benzoic acid and salicylic acid both affect in variable manner enhancing certain processes and inhibiting others (Raskin, 1992). Benzoic acid plays an important role in different physiological processes in plants such as growth, photosynthesis, nitrate

metabolism, ethylene and flowering production (Hayat *et al.*, 2010) and also provides protection against biotic and abiotic stresses such as heat (Kaya *et al.*, 2002). Benzoic acid application increased resistance against lipid peroxidation and membrane permeability in abiotic stress conditions (Horvath *et al.*, 2007). The protective action of benzoic acid includes the development of anti-stress programs and acceleration of normalization of growth processes after removal of stress factors (Sakhabutdinova *et al.*, 2003). Benzoic acid increased growth and yield (Christen and Lovett, 1993) and 1000-grain weight in barley; this may be attributed to the role of BZA in improving gas exchange ability that protects from damage (Anjum *et al.*, 2013). Benzoic acid foliar treatment resulted in improved water use efficiency 8.83%, intercellular CO₂ 3.11%, stomatal conductance 7.57%, transpiration rate 7.24%, ambient CO₂ ratio 6.78% and photosynthesis 11.54% (Anjum *et al.*, 2013).

Application of BZA increased chlorophyll contents under stress environment which ultimately improved survival in stress condition (Anjum *et al.*, 2013). These benzoic acid induced beneficial effects in gas exchange ultimately resulted in improved plant height, stem diameter, biological yield, grain yield and harvest index in water-stressed soybean plants. Sadak and Abd Elhamid, (2013) reported that exogenous application of benzoic acid increases the photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, chlorophyll *a+b*, carotenoids and subsequently to total pigments).

Foliar benzoic acid mediated regulations in agronomic, cell membrane thermostability and chlorophyll were associated with quality and yield attributes of cotton are scarce in the above-mentioned studies. Benzoic acid being regulator and its optimization as foliar application may prove a potent tool to alleviate terminal heat stress on cotton crop. Therefore, present experiment was conducted with the following objectives (i) To check the heat stress at squaring stage and its effect on cotton crop (ii) To adjust the foliar dose of benzoic acid as potential modulator of agronomic, physiological and quality traits of cotton under terminal heat stress.

2. Materials and methods:

2.1. Plant material:

Seed of genotype MNH-886 was collected from Cotton Research Station Multan, Pakistan for this study.

2.2. Experimental site:

The experiment was conducted at Agronomic Research Area, University of Agriculture Faisalabad, Pakistan. The site is geographically located at 73° east longitude, 31° north latitude, and at altitude of 184.4 meter.

2.3. Physiochemical traits of experimental site:

Soil samples were randomly collected from experimental plots with the help of soil auger. Samples were collected from the depths of 0-15 and 15-30 cm and analyzed to quantify different physiochemical attributes (ICARDA, 2013) (Figure 1).

2.4. Weather elements:

Weather elements of the experimental site measured during growing season over two years study are presented graphically (Figure 2). The experimental site was semiarid with annual mean rain fall of 375 mm. Temperature during the heat imposition is given in table 1.

2.5. Treatments:

The experiment was comprised of two variables a) heat stress i.e., of H_0 = No heat stress (control); H_1 = Heat stress at squaring for 7 days and b) varying concentrations of foliar benzoic acid Control, Water Spray, $BZA_{0.25}$ = 0.25, $BZA_{0.50}$ = 0.50, $BZA_{0.75}$ = 0.75 and $BZA_{1.00}$ = 1.00 mM.

2.6. Experimental design:

The experiment was laid out in randomized complete block design (RCBD) with split treatment structure having three blocks.

2.7. Imposition of treatments:

Ten plants in each experimental unit were tagged and observed regularly for initiation of squaring. When squaring was started, heat stress was applied using transparent and stabbed polythene sheet (Shakoor *et al.*, 2017; Kamal *et al.*, 2017a). Control (no heat stress imposition) plot was maintained under ambient environment. Temperature of all main plots was recorded three times a day and averaged. Temperature was observed by using digital meter (Digital Multimeter 50302). Benzoic acid is easily dissolved in water and was foliar applied as per treatment during heat stress imposition. The source also available with the name of benzoic acid.

2.8. Statistical analysis:

Analysis of variance was employed to determine significance (F-test) of heat stress and foliar benzoic acid. While, means of treatments were compared using Tukey's HSD (Honestly Significant Difference) test (Steel *et al.*, 1997).

2.9. Agronomic practices:

The crop was planted with manual dibbling having 75 cm apart ridges and plant to plant 30 cm distance. The seed rate during sowing was 10 kg ha⁻¹. Each plot size was 4.5 m × 3 m area. Fertilizer was applied at the rate of N: P: K @ 200: 115: 95 kg ha⁻¹. One fourth of the nitrogen and complete doses of the phosphorus and potash was applied at the time of planting of crop; whereas remaining nitrogen was applied in three equal splits, 1/4 at 30-35 days after sowing, 1/4 at squaring stage and 1/4 at boll formation stage uniformly in all experimental units. The crop was irrigated nine times according to requirement of the crop. Weeds were controlled by using one post emergence broad spectrum herbicide (Roundup (Glyphosate) at 3000 mL ha⁻¹) using shield (90 days after sowing) as well as two manual hoeing i.e. squaring (35 days after planting) and at flowering (60 days after planting) while the sucking insects and boll worms were controlled with insecticides. All other agronomic practices were kept normal and uniform for all the treatments.

2.10. Observations recorded:

Number of opened and unopened bolls at first and second picking of selected plants and total number of bolls were noted by totaling the opened and unopened bolls. Plant height (cm) at harvest of ten selected plants was measured with the help of meter rod at the time of second picking and average calculated. Average boll weight (g) was calculated by dividing the total seed cotton yield per plant by the number of opened bolls of the selected plants and then average was taken. Seed index (100-seed weight) was calculated by weighing the 100 seeds through electrical balance in grams from each of the selected plants and then average was taken. Seed cotton yield per hectare in kg was calculated from the seed cotton yield per plot. Relative Water Content (RWC) was calculated by using formula described by Barrs and Weatherly (1962).

$$\text{Relative water content (RWC) \%} = \frac{W_f - W_d}{W_s - W_d} \times 100$$

Total chlorophyll content was measured by using chlorophyll tester (CT- 102) at peak vegetative stage. Cell membrane thermo stability Cell membrane thermo stability was

measured at the peak flowering stage from the 20-22 days old fully expanded youngest leaves (Anderson *et al.*, 1990). RCI% an indicator of CMT, was calculated using the following formula (Sullivan, 1972)

$$\text{Relative cell injury (RCI) \%} = 1 - \left[\frac{1 - (T1/T2)}{1 - (C1/C2)} \right] \times 100$$

Seed cotton weight of 100 g was processed in a roller ginning unit and computed ginning out turn using formula (Singh, 2004).

$$\text{GOT (\%)} = \frac{\text{Lint weight}}{\text{Seed weight}} \times 100$$

Fiber fineness, uniformity, elongation, length and strength are quantified by processing lint in high volume instruments (HVI) Module-920 of HVI-900A.

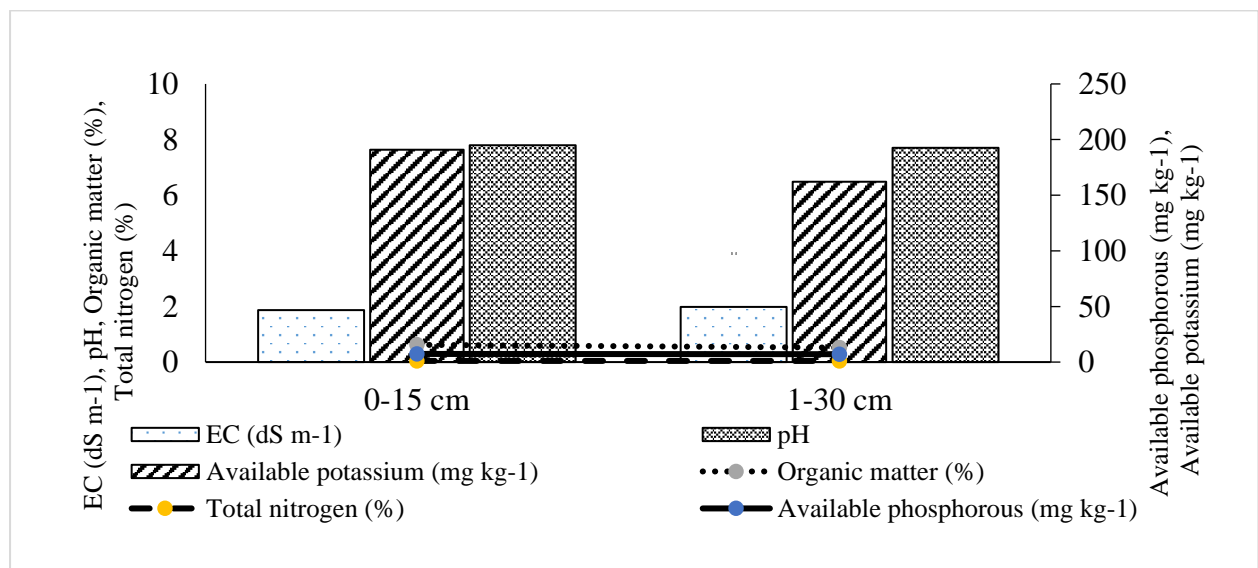


Figure 1. Physiochemical analyses of experimental site

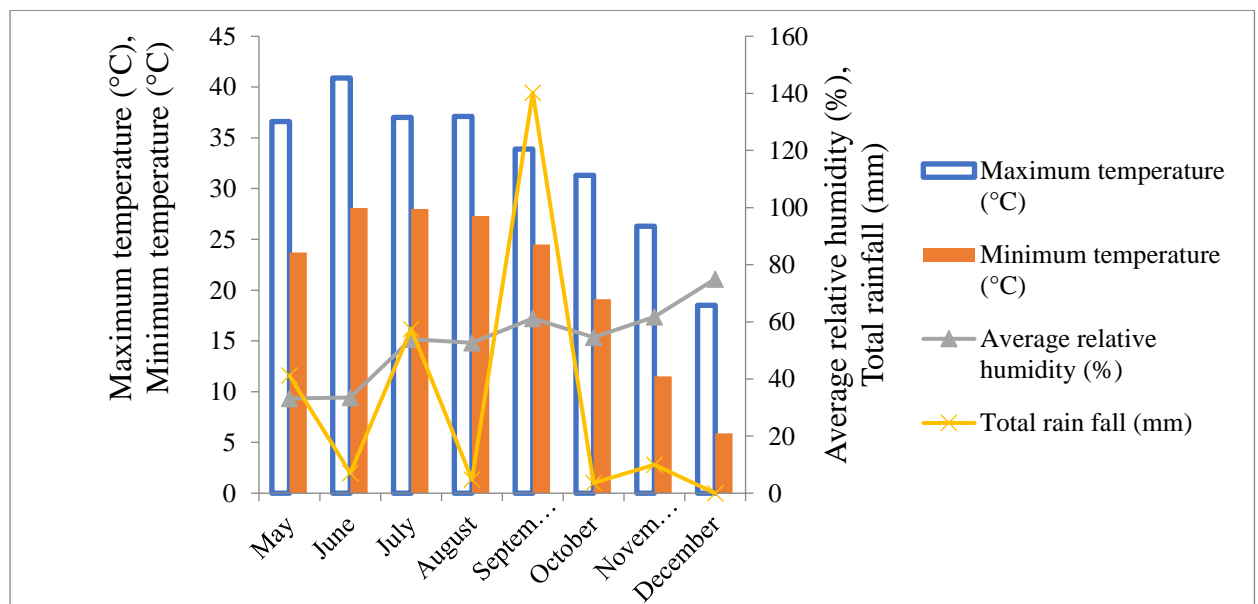


Figure 2. Weather elements of experimental site during growing season of cotton

3. Results:

3.1. Agronomic traits

In cotton production number of opened bolls per plant is an important yield component; more the number of opened bolls produced higher will be the seed cotton yield. Heat stress (H) and benzoic acid spray (F) significantly affected the number of opened, unopened, total bolls per plant and interactive effect of heat stress and benzoic acid spray was non-significant.

More number of opened, unopened and total bolls per plant were found in no heat stress situation and less were recorded in heat stress condition. Benzoic acid treatments, maximum opened and total bolls per plant were recorded where we applied 0.75 mM benzoic acid spray that was statistically at par where we applied 1.00 mM and 0.50 mM benzoic acid spray, respectively. In case of unopened bolls maximum were recorded with 0.50 mM benzoic acid spray and least number of opened, unopened and total bolls per plant were found in control treatment where no spray was applied (Table 2).

Cotton plants gained maximum height (140.1 cm) and seed index (7.53 g) when grown under no heat stress and foliar sprayed with 0.75 mM benzoic acid however; it was statistically at par with all other foliar spray levels (0.25, 0.50 and 1.00 mM benzoic acid) for both traits. While heat stressed cotton plants recorded maximum height (138.4 cm) when crop was sprayed with 1.00 mM benzoic acid and it was statistically not different from 0.75 mM benzoic acid spray. Under both heat and non-heat conditions control (no spray) remained at bottom followed by water spray with respect to plant height (Table 3).

Seed cotton yield was interactively effected by heat stress and benzoic acid spray. However, maximum seed cotton yield per ha ($3625.8 \text{ kg ha}^{-1}$) was recorded with 0.75 mM benzoic acid spray that was at par with 1.00 mM benzoic spray and minimum in no spray. In heat stress condition, maximum seed cotton yield ($2816.9 \text{ kg ha}^{-1}$) was recorded in 0.75 mM benzoic acid spray, that was statistically similar with 0.50 mM and 1.00 mM benzoic acid spray, respectively and minimum seed cotton yield per ha was recorded in no spray (Table 3).

3.2. Physiological attributes

Relative leaf water content, chlorophyll contents and cell membrane thermo stability were significantly affected by heat stress and benzoic acid spray but the interactive effect was non-significant. Maximum relative leaf water contents and chlorophyll contents were observed with 0.75 mM which was statistically at par with 1.00 mM, 0.50 mM and 0.25 mM and lowest where no spray was applied (Table 2).

No heat stress condition gave more cell membrane thermostability (50.09) than the heat stress condition (42.29). Maximum value of cell membrane thermostability (57.73) was recorded in where no spray which was statistically at par with water spray and 0.50 mM benzoic acid spray, respectively and minimum membrane thermostability (32.95) where we applied 1.00 mM benzoic acid spray (Table 2).

3.3. Fiber quality parameters

Heat stress significantly reduced the ginning out turn (GOT), fiber fineness (FF), fiber uniformity (FU), fiber elongation (FE), fiber length (FL) and fiber strength (FS) of cotton over control (no heat). However, application of BZA improved the GOT, FF, FU, FE, FL and FS of cotton over control and under heat stress.

Application of benzoic acid spray, maximum ginning out turn (38.92%) and fiber fineness (4.85 micronaire) were noticed with 0.75 mM which was statistically at par with 1.00 mM for GOT and also 0.50 mM for FF but significantly different from all other levels of benzoic acid spray including water spray and control (no spray) in table 2 and 4.

Benzoic acid spray showed maximum FU, FE, FL and FS with 0.75 mM level which were statistically similar with all other levels of benzoic acid and minimum values of these traits were recorded in control with no spray.

4. Discussion:

Early sowed cotton produced more bolls due to better climatic circumstances than late planted cotton due to heat stress (Bozбек *et al.*, 2006; Wrather *et al.*, 2008). High temperatures during anthesis are likely to cause pollination and/or fertilisation failure, resulting in reduced boll set. According to studies, high temperatures damage micro and mega sporogenesis, resulting in lower fruit set (Young *et al.*, 2004). Benzoic acid, sulfosalicylic acid and methyl salicylic acid are effective including heat, drought and chilling stress tolerance similar to salicylic and acetylsalicylic acids and increases the yield components such as total number of bolls, opened bolls and seed cotton yield per plant (Senaratna *et al.*, 2003). Cotton response to exogenous application of PGR such as benzoic acid hastened maturity and increased boll retention lower on the plant as well as delayed the boll opening and increased the boll weight (Nuti *et al.*, 2006), along with varying yield responses (Nuti *et al.*, 2006).

Reduction in boll weight was recorded in late planted cotton due to high temperature (Nehra and Matish Chandra, 2001). Boll weight in cotton was increased up to 25°C temperature then decreased gradually, the upper limit for boll existence is 32°C (Reddy *et al.*,

1999). Plant growth regulators have the ability to increase photosynthetic activity and dry matter concentration depending on the increase of photosynthetic pigments (Wu *et al.*, 1994). This process increased boll formation and boll weight in cotton (Sawan *et al.*, 2006). Increase in seed index was due to increased photosynthetic activity under heat stress environments (Biles and Cothren, 2001). Heat stress dislocated the uptake of water, ions and organic solutes across the membrane of plants inhibiting the photosynthesis and respiration, resulting in closure of stomata and shortage of relative water content of the plant leaf tissue (Wahid *et al.*, 2007). Foliar application of plant growth regulators on Japanese mint increased relative water content by about 59-75% (Mathur *et al.*, 2005).

Chlorophyll contents, carotenoids and chlorophyll *a*, *b* proportions decreased under heat and drought conditions (El- Tayeb, 2005). Chlorophyll solidity is a function of prevailing environmental temperature in cotton (Ananthi *et al.*, 2013). Foliar application of mepiquat chloride, benzyl adenine (BA), benzoic acid, ethephon and the combination of these plant growth regulators in cotton enhanced the leaf carotenoids and chlorophyll contents (Abed *et al.*, 2001).

Temperature controls the growth, development, phenology and adaptability of the cotton crop (Reddy *et al.*, 1991). When temperature exceeds than the optimum temperature (30/22-35/27°C, day/night) it affects the photosynthesis of the cotton crop as it is a C-3 plant. Exposure of the cotton crop to >40/32°C (day/night temperature) even for a short period of time at any growth stage affected the crop photosynthesis badly and reduced the seed cotton yield drastically (Azhar *et al.*, 2009). This lower value of CMT at late sowing date was due to higher temperature and higher relative humidity at peak flowering. High temperature changes the plant physiological processes (Downton and Slatyer, 1972). It affects the enzyme activity; changes the membrane structure and composition by weakening the hydrogen bonds and the interaction between the poles of the protein in the liquid medium of the membrane. So, the permeability of the membranes changes, leakage of the electrolytes from the membrane which results in reduction in yield (McDaniel, 1982). Cell membrane thermostability (CMT) was first time used by Sullivan (1972) for identifying the heat resistant and heat sensitive genotypes of the sorghum.

The loss in yield attributes due to improper coordination of source and sink organs may be linked to altered sucrose synthase activities during heat stress (Zhang *et al.*, 2018; Saleem *et al.*, 2020). Furthermore, heat stress causes a decrease in photosynthate accumulation, which reduces cotton yield-contributing qualities (Reda and Mandoura, 2011).

The use of plant growth regulators such as benzoic acid is an effective tool for increasing seed cotton yield, which can be attributed to plant water status, higher proline accumulation, reducing sugar, increased chlorophyll content, and cotton plant peroxidase activity. Plant growth regulators like mepiquat chloride and PGR-1V increased the number of bolls, opened bolls, and seed cotton production (Stephan and Cothren, 2001). In the current investigation, heat stress reduced the fibre quality features of cotton (Table 4). Previous research has shown that heat stress affects the key quality parameters of cotton, including GOT, FF, FU, FE, FL, and FS (Bozorov *et al.*, 2018). Heat stress during the reproductive stage reduces photosynthetic rate and impairs photosynthate transfer to growing bolls, reducing lint quality (Saleem *et al.*, 2020). Furthermore, heat stress-induced lipid peroxidation reduces fibre quality (Tables 4), as cotton fibres are cellular extensions of cotton seed (Zhi *et al.*, 2016). Furthermore, increasing duration of distinct phenophases may have facilitated assimilate translocation and accumulation in developing bolls, thereby improving fibre quality.

5. Conclusions

Deterioration in quality traits and reduction in seed cotton yield is due to considerable reduction in agronomic traits, were observed under 'heat at squaring for a week compared with 'no heat stress'. More damaging responses under 'heat at squaring for a week showed from the results of this experiment. Conversely, significant improvements in agronomic, yield, physiological and quality traits were found with the foliar application of BZA at varying doses. Foliar applied benzoic acid helped in ameliorating these negative impacts; 0.75 mM benzoic acid proved best in this regard.

Acknowledgments:

The authors are highly thankful to the Analytical Laboratory, Department of Agronomy University of Agriculture Faisalabad for technical support and laboratory facilities.

References:

- Abed, A.M. 2001. Growth and yield of cotton plant as affected by Pix, BA, Prep and their combinations. *Anls of Agri. Sci. Moshtohor.* 39:1551-1569.
- Ahmad, S., Q. Abbas, G. Abbas, Z. Fatima, A.U. Rehman, S. Naz, H. Younis, R.J. Khan, W. Nasim, M.H.U. Rehman, A. Ahmad, G. Rasul, M.A. Khan and M. Hasanuzzaman. 2017 Quantification of climate warming and crop management impacts on cotton phenology. *Plan Theory* 6:1-16.
- Ananthi, K., H. Vijayarghavan, M. Karuppaiya and T. Anand. 2013. Drought induced changes in chlorophyll stability index, relative water content and yield of cotton genotype. *Insight Botany*, 3:1-5.

- Anderson, J., G. McCollum and W. Roberts. 1990. High temperature acclimation in pepper leaves. Hort. Sci. 25:1272-1274.
- Anjum, S.A., E. Ullah, L. Xue, L. Wang, M.F. Saleem and C.J. Huang. 2013. Exogenous benzoic acid (BZA) treatment can induce drought tolerance in soybean plants by improving gas-exchange and chlorophyll contents. Aus. J. Crop Sci. 7:555-560.
- Azhar, F.M., Z. Ali, M.M. Akhtar, A.A. Khan and R. Trethowan. 2009. Genetic variability of heat tolerance, and its effect on yield and fiber quality traits in upland cotton (*Gossypium hirsutum* L.). Plant Breed. 128: 356-362. doi: 10.1111/j.1439-0523.2008.01574.x
- Barrs, H.D. and P.E. Weatherley. 1962. A reexamination of the relative turgidity techniques for estimating water deficit in leaves. Aust. J. Biol. Sci. 15:413-428.
- Biles, S.P. and J.T. Cothren. 2001. Flowering and yield response of cotton to application of Mepiquat Chloride and PGR-IV. Crop Science 41:1834-1837.
- Bozbek, T., V. Sezner and A. Unay. 2006. The effect of sowing date and planting density on cotton yield. Agron. J. 5:122-125.
- Bozorov, T.A., R.M. Usmanov, Y. Honglan, S.A. Hamdullaev, S. Musayev, J. Shavkiev, S. Nabiev, Z. Daoyuan and A.A. Abdullaev. 2018. Effect of water deficiency on relationships between metabolism, physiology, biomass, and yield of upland cotton (*Gossypium hirsutum* L.). J. Arid Land 10:441-456. doi: <https://doi.org/10.1007/s40333-018-0009-y>.
- Demmig-Adams, B., J.J. Stewart, R.B. Christopher and W.W. Adams. 2018. Optimization of photosynthetic productivity in contrasting environments by regulons controlling plant form and function. Int. J. Mol. Sci. 19:872.
- Downton, J. and R.O. Slatyer. 1972. Temperature dependence of photosynthesis in cotton. Plant Physiol. p. 518-522. Full Text via Cross Ref.
- El-Tayeb, M.A. 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regul. 45:215-224.
- Govt. of Pakistan. 2023. Economic survey of Pakistan 2022-23. Ministry of Food and Agriculture Islamabad, Pakistan, Chap. 2 p.17-41.
- Hayat, Q., S. Hayat, M. Irfan and A. Ahmad. 2010. Effect of exogenous salicylic acid under changing environment: A review. Environ. Exp. Bot. 68:14-25.
- Horvath, E., G. Szalai and T. Janda. 2007. Induction of abiotic stress tolerance by salicylic acid signaling. J. Plant Growth Regul. 26:290-300.
- ICARDA (International Center for Agricultural Research in the Dry Areas) (2013) Methods of soil, plant and water analysis: a manual for West Asia and North Africa region. In: Estefan G, Sommer R, Ryan J (eds) International Center for Agricultural Research in the Dry Areas.
- IPCC (Intergovernmental Panel on Climate Change). 2014. Technical summary of climate change 2014: Mitigation of climate change. Working group III contribution to the IPCC fifth assessment report (AR5).
- IPCC (Intergovernmental Panel on Climate Change). 2018. IPCC press release. IPCC sends governments Final Draft of Special Report on Global Warming of 1.5-C. p.1-2.
- Kamal, M.A., M.F. Saleem, M. Shahid, M. Awais, H.Z. Khan and K. Ahmed. 2017a. Ascorbic acid triggered physiochemical transformations at different phenological stages of heat-stressed Bt cotton. J. Agro. Crop Sci. 203:323-331.
- Kaya, C., H. Kirnak, D. Higgs and K. Saltali. 2002. Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high salinity. Sci. Hort. 93:65-74.

- Khan, H.Z.K., M.A. Shabir, N. Akbar, A. Iqbal, M. Shahid, A. Shakoor and M. Sohail. 2017. Effect of different tillage techniques on productivity of wheat (*Triticum aestivum* L.). *J. Agric. Basic Sci.* 2:44-49.
- Mathur, P., A.K.A. Farooqi and S. Srikant. 2005. Amelionative effective of chlormequat chloride on water stressed cultivars of Japanese mint (*Mentha avensis*). *Indian J. Pl. Physiol.* 10:41-47.
- McDaniel, R.G. 1982. The physiology of temperature effects on plants. *In: Christiansen, M.N., Lewis, C.F. (Eds.). Breeding Plants for Less Favourable Environments.* Wiley, New York, p. 13-45.
- Nehra, P.L. and M. Chandra. 2001. Performance of hirsutum cotton under different sowing dates and spacing. *J. Cotton Res. Devel.* 15:147-150.
- Nuti, R.C., R.P. Viator, S.N. Casteel, K.L. Edmisten and R. Wells. 2006. Effect of planting date, mepiquat chloride, and glyphosate application to glyphosate-resistant cotton. *Agron. J.* 98:1627-1633.
- Pettigrew, W.T. 2008. The effect of higher temperatures on cotton lint yield production and fiber quality. *Crop Sci.* 48:278-285
- Raskin, I., 1992. Role of salicylic acid in plants. *Annual Rev. Plant Physiol. Mole. Biol.* 43:439-463.
- Reda. F. and H.M.H. Mandoura. 2011. Response of enzymes activities, photosynthetic pigments, proline to low or high temperature stressed wheat plant (*Triticum aestivum* L.) in the presence or absence of exogenous proline or cysteine. *Int. J. Acad. Res.* 3:108-115.
- Reddy, K.R., G.H. Davidonis, A.S. Johnson and B.T. Vinyard. 1999. Temperature regime and carbon dioxide enrichment alter cotton boll development and fiber properties. *Agron. J.* 9:851-858.
- Reddy, V.R., D.N. Baker and H.F. Hodges. 1991. Temperature effects on cotton canopy growth, photosynthesis and respiration. *Agron. J.* 83:699-704.
- Roberts, E.M., N.R. Rao, J.Y. Huang, N.L. Trolinder and C.H. Haigler. 1992. Effects of cyclic temperatures on fiber metabolism in cultured cotton ovules. *Plant Physiol.* 100:979-986.
- Sadak, M. Sh. and E.M. Abd Elhamid. 2013. Physiological response of flax cultivars to the effect of salinity and salicylic acid. *J. Applied Sci. Res.* 9: 3573-3581.
- Sakhabutdinova, A.R., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova. 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol.* p. 314-319.
- Saleem, M.F., A. Shakoor, M. Shahid, M.A. Cheema, A. Shakeel, M. Shahid, M.U. Tahir and M.F. Bilal. 2018b. Removal of early fruit branches as potential regulator of Cry1Ac, antioxidants, senescence and yield in BT Cotton. *Ind. Crop Prod.* 124:885-898.
- Saleem, M.F., M. Shahid, A. Shakoor, M.A. Wahid, S.A. Anjum and M. Awais. 2018a. Removal of early fruit branches triggered regulations in senescence, boll attributes and yield of BT cotton genotypes. *Ann. Appl. Biol.* 172:224-235.
- Saleem, M.F., M.A. Kamal, M. Shahid, M. Awis, A. Saleem, M.A.S. Raza and B.L. Ma. 2020. Studying the foliar selenium-modulated dynamics in phenology and quality of terminal heat-stressed cotton (*Gossypium hirsutum* L.) in association with yield. *Plant Biosys.* pp. 1-11.
- Saleem, M.F., M.A. Kamal, S.A. Anjum, M. Shahid, M. Raza and M. Awais. 2018c. Improving the performance of BT-cotton under heat stress by foliar application of selenium. *J. Plant Nutr.* 41:1711-1723. doi. <https://doi.org/10.1080/01904167.2018.1459694>.

- Sarwar, M., M.F. Saleem, N. Ullah, S. Ali, M. Rizwan, M.R. Shahid and P. Ahmad. 2019. Role of mineral nutrition in alleviation of heat stress in cotton plants grown in glasshouse and field conditions. *Sci. Rep.* 9:13022. doi.org/10.1038/s41598-019-49404-6.
- Sawan, Z.M., H.M. Mahmoud and A.H. El-Guibali. 2006. Response of yield, yield components, and fiber properties of Egyptian cotton (*Gossypium barbadense* L.) to nitrogen fertilization and foliar-applied potassium and mepiquat chloride. *The J. Cotton Sci.* 10:224-234.
- Senaratna, T., D. Merritt, K. Dixon, E. Bunn, D. Touchell and K. Sivasithamparam. 2003. Benzoic acid may act as the functional group in salicylic acid and derivatives in the induction of multiple stress tolerance in plants. *Plant Growth Regul.* 39:71-81.
- Shakoor, A., M.F. Saleem, S.A. Anjum, M.A. Wahid and M.T. Saeed. 2017. Effect of heat stress and foliar applied benzoic acid on earliness and nutrients' uptake in cotton. *J. Agri. Res.* 55:15-28.
- Singh, P. 2004. Cotton breeding. Kalyani Publishers Ludhiana New Dehli Nodia (U.P.) Hyderabad Chennai Kolkata Cuttack India. pp. 118-295.
- Steel, R.G.D., J.H. Torrie and D. Dickey. 1997. Principles and procedures of statistics, a biometrical approach. 3rd ed. New York (NY): McGraw Hill Book Co. Inc.; p.352-358.
- Stephan, B. and J.T. Cothren. 2001. Flowering and yield response of cotton to application of mepiquat chloride and PGR-IV. *Crop Sic.* 41:1834-1837.
- Sullivan, C.Y. 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. *In: N.P. Rao, and L.R. House (Eds.). Sorghum in Seventies*, 247. Oxford and IBH Publ. Co, New Delhi.
- Szymańska, R., I. Ślesak, A. Orzechowska and J. Kruk. 2017. Physiological and biochemical responses to high light and temperature stress in plants. *Environ. Exp. Bot.* 139:165-177.
- Tonne, P. 2011. Cotton and climate change: impacts and options to mitigate and adapt. ITC, 2011. Technical paper, Doc. No. MAR-11-200.E Technical Report, Geneva, xii, 32 p
- USDA, 2018. Pakistan likely to be a big cotton importer, Islamabad. Global agriculture information network.
- Wahid, A., S. Gelani, M. Ashraf and M. Foolad. 2007. Heat tolerance in plants: an overview. *Environ. Exp. Bot.* 61:199-223.
- Wrather, J.A., B.J. Phipps, W.E. Stevens, A.S. Phillips and E.D. Vories. 2008. Cotton planting date and plant population effect on yield and fiber quality in the Mississippi Delta. *J. Cotton Sci.* 12:1-7.
- Wu, Z.L., Q.B. Pan, Y.H. Gao, J.Y. Wang and J. Wang. 1994. Technical researches on the all-round chemical regulation of cotton plants. *China Cottons* 21:10-11.
- Young, L.W., R.W. Wilen and P.C. Bonham-Smith. 2004. High temperature stress of (*Brassica napus*) during flowering reduces micro and mega gametophyte fertility, induces fruit abortion, and disrupts seed production. *J. Exp. Bot.* 55:485-495.
- Zhang, C.X., B.H. Feng, T.T. Chen, W.M. Fu, H.B. Li, G.Y. Li, Q.Y. Jin, L.X. Tao and G.F. Fu. 2018. Heat stress-reduced kernel weight in rice at anthesis is associated with impaired source-sink relationship and sugars allocation. *Environ. Exp. Bot.* 155:718-733. doi.org/10.1016/j.envexpbot.2018.08.021.
- Zhi, X.Y., Han, Y.C., Li, Y.B., Wang, G.P., Du, W.L., Li, X.X., Mao, S.C. and Lu, F. 2016. Effects of plant density on cotton yield components and quality. *J. Integrat. Agri.* 15:1469-1479.

Table 1: Varying mean temperatures (°C) during heat imposition

Heat stress	July 28	July 29	July 30	July 31	August 01	August 02	August 03
No heat imposition (H ₀)	38.6	41.2	40.4	38.7	40.0	41.2	37.6
Heat stress at squaring for 7 days (H ₁)	-	-	-	-	45.3	47.4	43.6

Latitude = 31° - 26'N; Longitude = 73° - 06'E; Altitude = 184.4 m

Table 2: Effect of foliar-applied benzoic acid on agronomic, physiological traits and ginning out turn (GOT) of heat-stressed cotton

Treatments	TB	OB	UOB	ABW	SI	GOT	RLWC	CC	CMT
Heat Stress (H)									
No heat stress (H ₀)	39.21 a	30.12 a	8.93 a	3.30 a	7.53 a	33.36 a	49.81 a	1.509 a	50.09 a
Heat stress at squaring (H ₁)	31.02 b	24.40 b	6.59 b	2.66 b	6.48 b	36.93 b	44.69 b	1.287 b	42.29 b
HSD (≤ 0.05)	6.888	4.854	0.234	0.230	0.726	1.902	4.382	0.0944	4.078
Benzoic acid levels (BZA)									
Control (No spray)	30.20 d	24.06 b	6.14 c	2.60 b	6.44 b	31.89 c	42.94 c	1.273 c	57.73 a
Control (Water spray)	32.64 cd	25.14 b	7.49 b	2.69 b	6.73 ab	33.27 bc	44.60 bc	1.328 bc	52.82 ab
0.25 mM Benzoic acid spray	34.50 bc	26.61 ab	7.89 b	3.00 ab	6.97 ab	34.68 bc	46.79 abc	1.416 ab	44.37 bc
0.50 mM Benzoic acid spray	37.39 ab	27.68 ab	9.72 a	3.05 ab	7.29 ab	35.70 b	48.83 ab	1.448 a	46.84 ab
0.75 mM Benzoic acid spray	39.59 a	31.24 a	8.15 b	3.46 a	7.64 a	38.92 a	51.27 a	1.469 a	42.45 bc
1.00 mM Benzoic acid spray	36.40 abc	28.79 ab	7.18 bc	3.04 ab	6.97 ab	36.41 ab	49.08 ab	1.456 a	32.95 c
HSD (≤ 0.05)	3.963	5.520	1.132	0.523	0.955	3.162	5.834	0.0984	13.046

Any two means not sharing a letter in common differ significantly at $p \leq 0.05$

NS = Non-significant, TB = Total bolls per plant, OB = Opened bolls per plant, UOB = Unopened bolls per plant

ABW = Average boll weight (g), SI = Seed index (g), GOT = Ginning out turn (%), RLWC = Relative leaf water contents (%)

CC = Chlorophyll contents, CMT = Cell membrane thermostability

Table 3: Effect of foliar-applied benzoic acid on plant height and seed cotton yield of heat-stressed cotton

Treatments	PH	SCY
Heat Stress (H)		
No heat stress (H ₀)	126.7	2952.7
Heat stress at squaring (H ₁)	118.9	2345.8
HSD (≤ 0.05)	12.39	99.61
Benzoic acid levels (BZA)		
Control (No spray)	112.6	2083.3
Control (Water spray)	115.3	2223.9
0.25 mM Benzoic acid spray	118.3	2547.6
0.50 mM Benzoic acid spray	123.6	2760.3
0.75 mM Benzoic acid spray	133.3	3221.3
1.00 mM Benzoic acid spray	133.7	3059.3
HSD (≤ 0.05)	11.46	229.46
Heat Stress \times Benzoic acid levels		
H ₀ \times Control (No spray)	118.6 b	2462.0 c
H ₀ \times Control (Water spray)	121.1 b	2571.3 c
H ₀ \times 0.25 mM BZA	124.9 ab	2766.1 bc
H ₀ \times 0.50 mM BZA	126.4 ab	2952.1 b
H ₀ \times 0.75 mM BZA	140.1 a	3625.8 a
H ₀ \times 1.00 mM BZA	128.9 ab	3338.9 a
H ₁ \times Control (No spray)	106.6 c	1704.5 d
H ₁ \times Control (Water spray)	109.4 c	1876.4 d
H ₁ \times 0.25 mM BZA	111.7 bc	2329.0 c
H ₁ \times 0.50 mM BZA	120.7 bc	2568.4 abc
H ₁ \times 0.75 mM BZA	126.4 ab	2816.9 a
H ₁ \times 1.00 mM BZA	138.4 a	2779.7 ab
HSD (≤ 0.05) Interaction	16.22	342.83

Means not sharing a letter in common within a row differ significantly at 5% probability.

NS = Non-significant, PH = Plant height (cm), SCY = Seed cotton yield per hectare (kg)

Table 4: Effect of foliar-applied benzoic acid on fiber quality parameters of heat-stressed cotton

Treatments	FF	FU	FE	FL	FS
Heat Stress (H)					
No heat stress (H ₀)	4.40	51.46	6.98	25.57	27.57
Heat stress at squaring (H ₁)	4.47	51.01	6.66	24.88	26.94
HSD (≤ 0.05)	NS	NS	NS	NS	NS
Benzoic acid levels (BZA)					
Control (No spray)	4.07 d	48.97 b	6.28 b	23.87 c	25.83 b
Control (Water spray)	4.14 cd	50.82 ab	6.49 ab	24.18 c	26.61 b
0.25 mM Benzoic acid spray	4.37 bcd	51.64 ab	6.83 ab	24.87 bc	27.23 ab
0.50 mM Benzoic acid spray	4.56 abc	51.96 ab	7.08 ab	26.03 ab	27.98 ab
0.75 mM Benzoic acid spray	4.85 a	53.32 a	7.28 a	26.98 a	28.80 a
1.00 mM Benzoic acid spray	4.64 ab	50.70 ab	6.97 ab	25.44 abc	27.06 ab
HSD (≤ 0.05)	0.438	3.391	0.908	1.652	2.194

Any two means not sharing a letter in common differ significantly at $p \leq 0.05$

NS = Non-significant, FF = Fiber fineness (micronaire), FU = Fiber uniformity (%), FE = Fiber elongation (%), FL = Fiber length (mm), FS = Fiber strength (g/tex)