A-6237
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RESEARCH ARTICLE Indian Journal of Agricultural Research

Unravelling the Role of Phytohormones against Heat Stress in Maize

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10.18805/IJARe.A-6237

ABSTRACT

Background: Drought stress is a significant challenge for maize production, causing significant harm to crop growth and yield. In this experiment two set of pot culture experiment was conducted with three replication to assess the effect of phytohormone foliar spray on maize COH(M) 8.

Methods: This experiment study was conducted at the Seed Science and Technology, TNAU in the summer season during 2022 and 2023, respectively. One set of potted plants were kept under ambient temperature (34±2°C). Another set of potted plants were raised at elevated temperature of 42°C in the Open Top Chamber. The plants were sprayed with sodium nitroprusside (50 µM and 75 µM), brassinolides (0.2 ppm and 0.5 ppm) and salicylic acid (50 ppm and 75 ppm) at 40 and 47 days after sowing along with control.

Result: Foliar spraying with sodium nitroprusside 50 µM and salicylic acid 75 ppm recorded higher plant height, early flowering, pollen viability, chlorophyll content and enzyme activities (catalase, peroxidase and superoxide dismutase) compared to the control plants. As a result, these findings suggest that use of sodium nitroprusside 50 µM enhances plant defence mechanisms against heat stress in maize.

Key words: Abiotic stress, Enzymes, Heat stress, Maize, Phytohormones.

INTRODUCTION

Heat stress is a major abiotic factor that reduces productivity and quality, leading to significant economic losses (Sharma and Manjeet, 2020). Heat stress has a significant negative impact on plant growth, development and yield (Youldash *et al.,* 2020). When temperatures surpass a particular level, which is typically approximately 30°C for the majority of crops (Sattar *et al.,* 2020) plants undergo changes such as reduced plant height, root growth, leaf size, uptake of water, nutrients (Ray *et al.*, 2012), photosynthesis activity, change of source-sink relationships (Rennenberg *et al.*, 2006), respiration, reduce chlorophyll content, and disrupt the balance of carbon assimilation that can affect their productivity (Tiwari *et al.,* 2019). Heat stress has a significant impact on physiological processes such as seed germination and vigor, leaf enlargement, root growth, photosynthesis, reproductive development, rate of filling and, grain filling duration ultimately reducing yield and grain quality. Different proteins, membranes, RNA species, and cytoskeleton structures are affected differently by heat stress. It also modifies the efficiency of enzymatic reactions within the cell, which causes a barrier to major physiological processes and metabolic imbalance (Suzuki *et al.,* 2011). Heat stress also causes an oxidative burst, which results in overproduction of ROS that cause membrane lipid peroxidation, DNA damage, protein oxidation, and cell apoptosis enzyme inactivation and macromolecule damage (Iqbal *et al.,* 2022; Choudhary *et al.,* 2020) as well as disruption of cell elongation and differentiation (Bazzaz *et al.,* 2020). Rubisco becomes less active or inactive at high temperatures, which leads to a reduction in the fixing of carbon dioxide.

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How to cite this article: Manonmani, V., Revathy, V., Ambika, S., Paramasivam, R., Kavitha, S., Nivethitha, M. and Umanath, M. (2024). Unravelling the Role of Phytohormones against Heat Stress in Maize. Indian Journal of Agricultural Research. doi: 10.18805/IJARe.A-6237.

Submitted: 04-04-2024 **Accepted:** 03-10-2024 **Online:** 12-11-2024

In maize during flowering and grain filling stage, temperature above 40°C will affect the productivity (Shiferaw *et al.*, 2011). The degree and duration of exposure also increases the effect on plant. Average optimum temperature is 30-34°C. Increase in temperature by 2°C reduces yield by 13%. Temperature above 35°C causes increased pollen sterility and therefore reduces the yield (Suwa *et al.*, 2010). High temperature negatively affects the reproductive stage by reducing the flower number, size and deformation of floral organs which results in reduction of the seed yield (Lobell *et al.*, 2011).

External application of various phytohormones like salicylic acid, brassinolide and sodium nitroprusside plays an important role in alleviating the heat stress by acting as messenger molecules and also plays a serious role in growth and development of plants. The hormonal cross talk coordinates the defence responses in plants to heat stress.

Foliar application of salicylic acid reduced the heat stress effects by membrane damage reduction through reduced leakage of electrolytes, malondialdehyde content and hydrogen peroxide content (Hameed and Ali, 2016). Salicylic acid (SA) is an endogenous plant growth regulator plays a vital role in plant growth, ion uptake, transport, interaction with other organisms and in the responses to environmental stress (Kuchlan and Kuchlan, 2023). Foliar application of brassinolides protects the plants through amplification responses to heat stress and saving the expression of plant growth promoters. Foliar application of sodium nitroprusside reduced heat stress by decreasing ion leakage and increasing the activity of anti-oxidant enzymes (Deng and Song, 2012) and also alleviate membrane lipid peroxidation damage under drought (Zhao *et al*., 2024). The effectiveness of exogenously applied phytohormones at optimum concentration for protecting the crop against high temperature needs to be studied.

MATERIALS AND METHODS

This experiment study was conducted at the Seed Science and Technology, TNAU in the summer season during 2022 and 2023, respectively. The crop was raised in the pots in two sets. One set of potted plants were kept under ambient temperature (34±2°C) for the total duration upto the flowering stage (Fig 1a and 1b). Another set of potted plants were raised at the ambient temperature until the completion of flowering stage. Then the plants were exposed to the elevated temperature of 42°C in the Open Top Chamber for 10 days during the flowering stage. Nutrients and plant protection management were carried out as per the recommendation of TNAU crop production guide (NPK @ 175:90:90 kg ha^{-1}). The crop was foliar sprayed with phytohormones using knapsack sprayer at two growth stages. The growth parameters (plant height) and physiological parameters (chlorophyll content (mg/g), relative water content (%), proline content (mg/g), catalase (µg of H_2O_2/g/minute), peroxidase activity (g tissue/min), superoxide dismutase (U mg^{1} protein min^{-1}) were observed and the leaf samples were collected before spray and 3 days after second foliar spray and analysed for the enzyme activity. The mean value was calculated. The significance of data from several studies was determined using the "F" test, as described by (Panse and Sukhatme, 1957). Treatment details are given in Table 1.

Fig 1 (a): View of pot culture experiment at the ambient condition (34±2°C).

Fig 1 (b): View of pot culture experiment in the Open Top Chamber (42°C).

Table 1: Treatment details.

Foliar spray with phytohormones	Concentration	Temperature stress	Stages of foliar spray
Control	-		-
Salicylic acid	50 ppm		
	75 ppm	Ambient condition	40 and 47 days
Brassinolides	0.2 ppm	(34±2°C)	after sowing
	0.5 ppm		
Sodium nitroprusside	50 µM	Open Top Chamber	
	75 µM	(42°C)	

RESULTS AND DISCUSSION

Heat stress is a critical concern for food crop yield in the changing climatic scenario and it is a great challenge for everyone in achieving food security (Chaturvedi *et al.*, 2017). Maize plants are vulnerable to heat stress (temperatures above 30°C) (Fig 2a and 2b).

Significant increase in the plant height was noticed due to foliar application of phytohormones. Among the foliar spraying treatments, maize plants sprayed with salicylic acid 75 ppm recorded the highest plant height of 185.5 cm and 188.6 cm which was on par with sodium nitroprusside 50 µM (180.7 cm and 185.6 cm at 60 and 90 DAS respectively whereas the lowest plant height was observed for the control which recorded 160.5 cm 164.3 cm at 60 and 90 DAS respectively (Table 2). Salicylic acid stimulated cell division in apical meristem in plants (Shakirova *et al.*, 2003). Foliar application of salicylic acid increased the plant growth in *Cicer arietinum* (Hayat *et al.*, 2012) and *Rosmarinus officinallis* (El-Esawi *et al.,* 2017). Salicylic acid controls the functions of plant in a concentration dependent manner where the lower concentrations of the salicylic acid induced the functions of the plant while higher concentrations inhibited the plant functions. Sodium nitroprusside significantly increased growth and its related parameters compared to the control at all stages of the crop growth (Hajihashemi *et al.,* 2021).

Flowering was delayed in the open top chamber (42!) when compared to the ambient condition (34°C). Plants foliar sprayed with sodium nitroprusside 50 µM and salicylic acid 75 ppm (52 days) flowered 4 days earlier when compared to control (56 days) at the ambient condition (34°C) (Table 2). Plants foliar sprayed with sodium nitroprusside 50 ìM and salicylic acid 75 ppm (54 days) flowered early by 4 days when compared to control (58 days) at the open top chamber (42°C). Days to 50% flowering was 55 days in plants foliar sprayed with sodium nitroprusside 50 µM which was on par with salicylic acid 75 ppm foliar spraying whereas control seeds have taken 58 days to attain 50% flowering at the ambient condition (34°C). The treatments such as sodium nitroprusside 50 µM and salicylic acid 75 ppm taken less days for 50% flowering compared to control at the open top chamber (42°C). Salicylic acid application enhanced the flowering (Aghdam *et al.,* 2016). Nitric oxide (NO), which SNP (sodium nitroprusside) is a donor of, has been demonstrated to be involved in the way plants react to environmental stressors, such as heat stress (Qiao and Fan, 2008). Application of SNP to plants can mitigate certain adverse consequences of thermal stress, such as diminishing oxidative stress, preserve the integrity of the plant cell membrane, enhancing photosynthesis, and elevating antioxidant activity and enzymes involved in stress signalling (Yu *et al.,* 2013; Siddiqui *et al.,* 2017a; Kharbech *et al.,* 2020).

The maximum pollen viability was observed in the plants foliar sprayed with sodium nitroprusside 50 ìM (88%) whereas the minimum was observed in control plants (83%) at the ambient condition (34°C) (Table 2). However, the pollen viability was reduced at the elevated temperature (42°C) in the open top chamber. Sodium nitroprusside 50 ìM (80%) applied plants recorded higher pollen viability when compared to control (68%). Pollen abortion caused by different stresses is always a result of their abnormal development of tapetum (Trost, 2014), which directly contacts the male gametophyte and plays a serious role in the development and maturation of microspores. Pollen viability, stigma receptivity and ovule viability was reduced in plants exposed to heat stress but increased with the exogenous application of sodium nitroprusside as a consequence of improvement in leaf and anther function to significantly increase physiological function and yield in heat stressed lentil (Esim and Atici 2014; Sita *et al.,* 2021).

The plants foliar sprayed with salicylic acid 75 ppm and sodium nitroprusside 50 µM registered maximum proline content (360 mg/g) when compared to control 320 mg/g at the ambient condition. Similar trend was observed for the plants kept at the elevated temperatures in the open top chamber. However, the proline content was increased when compared to the plants kept at the ambient condition.

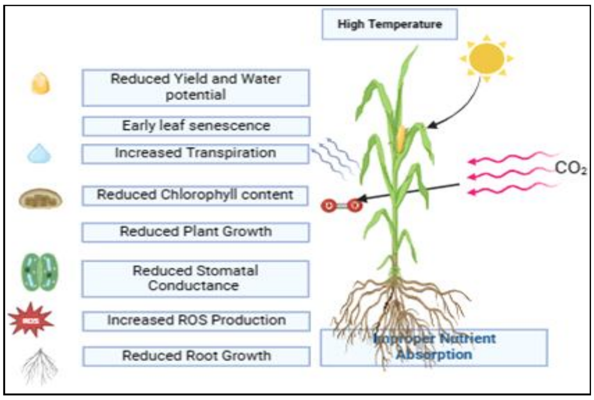

Fig 2 (a): Effect of heat stress on maize plant.

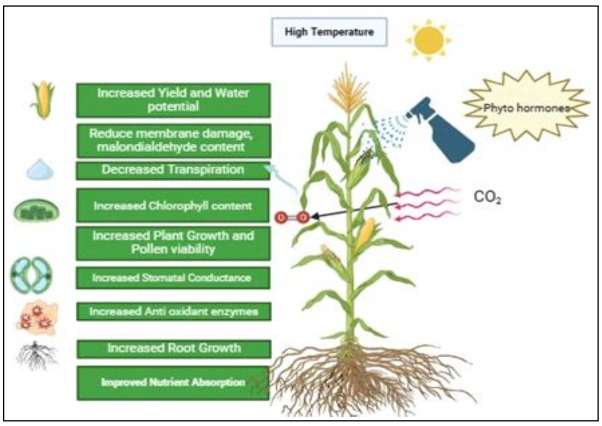

Fig 2 (b): Influence of phytohormones spray on maize plant under heat stress.

The plants foliar sprayed with sodium nitroprusside 50 ìM registered maximum proline content (420) followed by salicylic acid 75 ppm (410). The minimum proline content was observed in control (340) (Fig 3). Aftab *et al.* (2010) reported that salicylic acid has been involved in regulation of essential plant biological processes like nitrogen and proline metabolism, photosynthesis, glycine betaine production, multiple antioxidant defense mechanism and water relations in plants under stress situations. Tufail *et al.* (2013) suggested that action of salicylic acid under heat stress increased the production of proline which improved the osmotic potential allowing the plant for increased water uptake which results in positive effect of on stomatal complex and photosynthetic process which leads to improved efficiency of photosystem a! and activity of Rubisco increases that collectively resulted in improved photosynthesis. Salicylic acid modifies both the osmolytes and metabolites production and also the nutrient status of the plants and plays defensive role under the abiotic stress situations (Ghani *et al.*, 2015).

The plants foliar sprayed with sodium nitroprusside 50 µM (0.947 mg/g) at 40 and 47 DAS recorded the maximum chlorophyll content over control in the open top chamber (Table 3). This was supported by Mittler (2002) that application of sodium nitroprusside led to higher levels of chlorophylls a and b due to less degradation of chlorophyll under heat stress. The application of sodium nitroprusside significantly improved chlorophyll contents and transpiration rate, which could be attributed to the inhibition of reactive oxygen species production or to maintaining photosynthesis stability under stress conditions (Tian *et al.,* 2015; Muthulakshmi *et al.,* 2017). Exogenous application of sodium nitroprusside treatment improved relative water content, leaf chlorophyll concentration, and electrolyte content in heat-stressed *Zingiber officinale* (Li *et al.,* 2013). The restoration of net photosynthetic rate (Haldimann and Feller, 2004) and recovery from chlorophyll bleaching were detected during sodium nitroprusside treatment by delayed reduction of photosynthetic pigment in *Quercus pubescens*, *Triticum aestivum*, *Vicia faba, Solanum Lycopersicon* and *Oryza sativa* (Yang *et al.,* 2011). Heat stress is accompanied by oxidative stress as noticed by the burst of hydrogen peroxide in cells after high temperature exposure thus reducing yield (Sharma *et al.,* 2020).

The present study proved that the plants foliar sprayed with salicylic acid 50 and 75 ppm recorded the maximum Relative Water Content (18%) (Table 3) at the ambient condition. Plants foliar sprayed with sodium nitroprusside 50 µM and salicylic acid 75 ppm (14%) registered increased relative water content in the open top chamber (42°C) when compared to control (11%). Similar findings were observed by Li *et al.* (2013) that exogenous application of sodium nitroprusside retrieved relative water content and chlorophyll concentration of leaf in heat-stressed *Zingiber officinale.*

The maximum catalase activity was observed in the leaves foliar sprayed with sodium nitroprusside 50 µM (127.3 µg of H_2O_2/g/minute) and minimum was observed in the control (121.0 µg of H_2O_2/g/minute) at the ambient condition (Table 4). In the open top chamber (42°C) the maximum catalase activity was observed with sodium nitroprusside 50 µM (132.1 µg of H_2O_2/g/minute) and minimum was observed in the control (123.2 µg of H_2O_2/g/minute). The peroxidase and superoxide dismutase activity was maximum for sodium nitroprusside 50 ìM applied plants (145.4 g tissue/min, 1.47, respectively) whereas the minimum peroxidase activity was observed in the control plants (127.3 g tissue/min, 1.12, respectively) at the ambient

Table 2: Effect of foliar spray with phytohormones on plant height (cm), days to first flowering and 50% flowering in maize COH (M) 8 under temperature stress.

Foliar spray treatments		Plant height		Ambient condition (34°C)		Open top chamber (42°C)		Ambient condition (34°C)	Open top chamber (42°C)
		60 DAS	90 DAS	Days to first flowering	Days to 50% flowering	Days to first flowering	Days to 50% flowering	Pollen viability (%)	Pollen viability (%)
Control		160.5	164.3	56	58	58	61	83 (65.65)	68 (55.55)
Salicylic acid	50 ppm	167.4	169.2	54	56	55	57	86 (68.03)	72 (58.05)
	75 ppm	185.5	188.6	52	55	54	56	87 (68.87)	75 (60.00)
Brassinolides	0.2 ppm	169.8	174.2	55	57	57	60	84 (66.42)	70 (56.79)
	0.5 ppm	166.4	168.6	54	57	57	59	85 (67.22)	73 (58.69)
Sodium	50 µM	180.7	185.6	52	55	54	56	88 (69.73)	80 (64.43)
nitroprusside	75 µM	172.5	175.6	53	56	55	57	80 (63.44)	74 (59.34)
Mean		171.828	175.16	53.857	56.428	55.714	58.00	84.714	73.143
SEd		1.566	2.622	0.743	0.617	0.859	0.817	1.036	0.767
CD (P=0.05)		3.291**	5.452**	1.545	1.284	1.788	1.699	2.154	1.595

condition (34°C). The plants foliar sprayed with sodium nitroprusside 50 µM registered maximum superoxide dismutase activity (1.60) followed by salicylic acid 75 ppm (1.40) and the minimum superoxide dismutase activity was observed in control (1.10) at open top chamber (Fig 4). By encouraging the expression of HSPs in plants, sodium nitroprusside can help them further resist heat stress (Garg *et al.,* 2012). Application of sodium nitroprusside (0.5 mM) increased enzymatic antioxidants and non-enzymatic antioxidants such as peroxidase, superoxide dismutase and catalase enzymes under heat stress (43°C) and reduced electrolyte leakage and lipid peroxidation in wheat

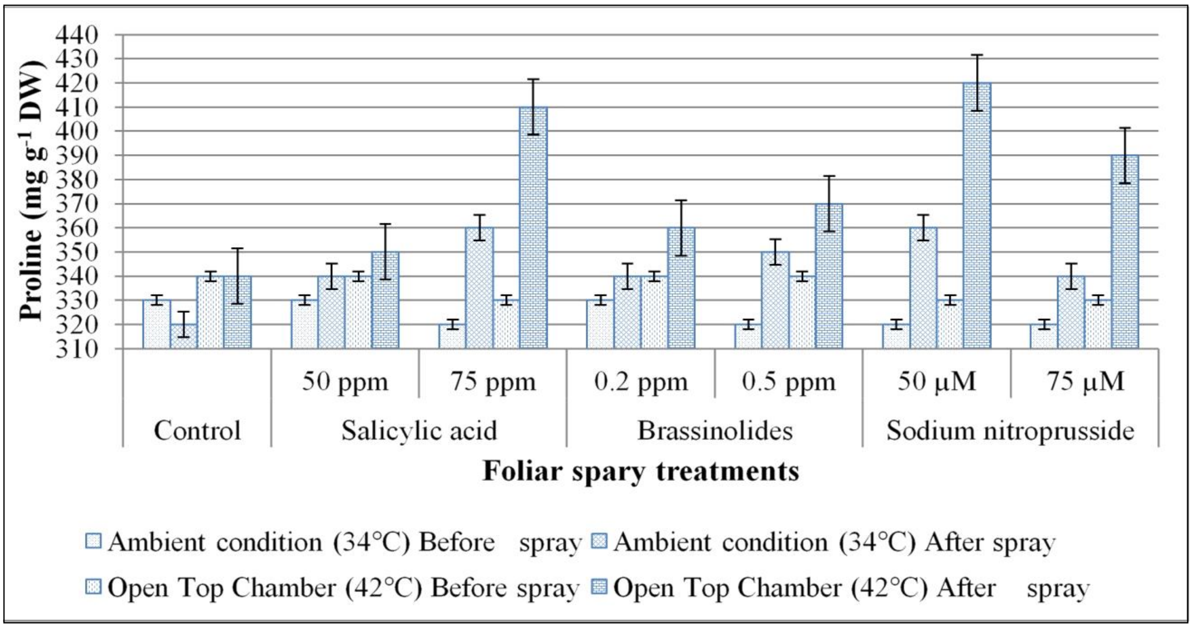

Fig 3: Effect of foliar spray with phytohormones on proline (mg/g DW) in maize COH (M) 8 under temperature stress.

Table 3: Effect of foliar spray with phytohormones on chlorophyll content (mg/g) in maize COH (M) 8 under temperature stress.

Foliar spray treatments		Ambient condition (34°C)			Open top chamber (42°C)			Ambient condition (34°C)	Open top Chamber (42°C)
		Chlorophy ll 'a'	Chlorophyl l 'b'	Total chlorophy ll	Chlorophy ll 'a'	Chlorophy ll 'b'	Total chlorophyll	Relative water content	
Control		0.356	0.312	0.738	0.335	0.539	0.869	16 (23.57)	11 (19.37)
Salicylic acid	50 ppm	0.375	0.441	0.743	0.302	0.601	0.896	18 (25.10)	13 (21.13)
	75 ppm	0.359	0.514	0.868	0.341	0.595	0.930	18 (25.10)	14 (21.97)
Brassinolides	0.2 ppm	0.336	0.626	0.955	0.338	0.512	0.845	17 (24.33)	13 (21.13)
	0.5 ppm	0.350	0.628	0.971	0.350	0.521	0.866	17 (24.35)	13 (21.13)
Sodium nitroprusside	50 µM	0.353	0.628	0.974	0.351	0.604	0.947	17 (24.35)	14 (21.97)
	75 µM	0.367	0.636	0.995	0.347	0.597	0.938	17 (24.35)	13 (21.13)
Mean		0.357	0.541	0.892	0.338	0.567	0.899	17.143	13.00
SEd		0.005	0.009	0.012	0.004	0.007	0.010	0.334	0.163
CD (P=0.05)		0.009	0.019	0.024	0.007	0.016	0.021	0.694	0.339

Table 4: Effect of foliar spray with phytohormones on catalase (µg of H_2O_2/g/minute) and peroxidase (g tissue/min) activity in maize COH (M) 8 under temperature stress.

Foliar spray treatments		Ambient condition (34°C) catalase		Open top chamber (42°C)		Ambient condition (34°C) peroxidase		Open top Chamber (42°C)	
		Before spray	After spray	Before spray	After spray	Before spray	After spray	Before spray	After spray
Control		120.3	121.0	115.6	123.2	126.2	127.4	125.4	127.3
Salicylic acid	50 ppm	119.5	123.9	114.4	126.6	128.9	129.3	127.3	135.1
	75 ppm	118.4	125.8	116.5	130.5	127.2	132.2	130.2	142.5
Brassinolides	0.2 ppm	119.3	122.9	115.4	125.9	126.3	127.4	126.4	138.2
	0.5 ppm	120.4	124.4	116.8	127.2	128.0	128.5	128.5	133.2
Sodium	50 µM	121.6	127.3	115.9	132.1	125.1	130.4	129.4	145.4
nitroprusside	75 µM	122.0	124.7	116.9	130.2	125.4	129.6	130.6	140.3
Mean		120.214	124.286	115.928	127.957	126.728	129.257	128.257	137.428
Ed		1.431	1.591	1.831	1.610	1.512	1.702	1.824	1.274
CD (P=0.05)		NS	3.308	NS	3.348	NS	NS	NS	2.650

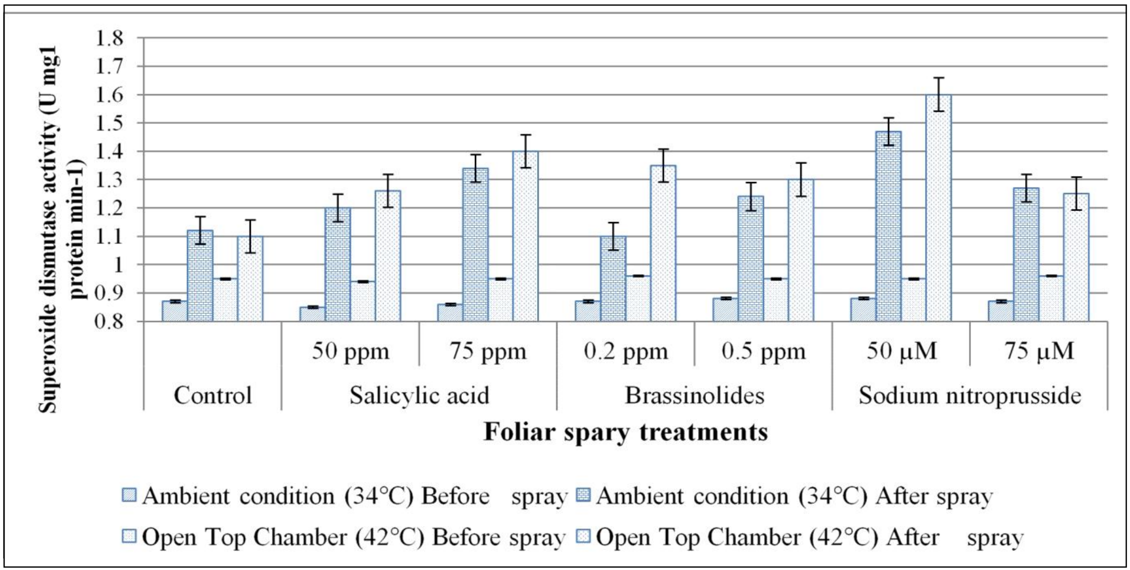

Fig 4: Effect of foliar spray with phytohormones on superoxide dismutase activity.
(U mg^{1} protein min^{-1}) in maize COH (M) 8 under temperature stress.

(Karpets *et al.*, 2011; Hasanuzzaman *et al.*, 2012). Mung bean leafs treated with sodium nitroprusside recorded lower membrane leakage, H2O2 production and lipid peroxidation (48%) compared to the control (Yang *et al.*, 2006). The same treatment increased the carbonic anhydrase, nitrate reductase and RuBisCo as well as osmolytes like glycine betaine and proline in tomato (Siddiqui *et al.*, 2017b). Under heat stress, paddy seedlings showed increased transcription of enzymes such as small heat shock protein 26, sucrose phosphate synthase and delta-1-pyrroline-5-carboxylate synthase (Uchida *et al.*, 2002). Sodium nitroprusside increased the H2S accumulation and activities of L-cysteine disulfhydrase, resulting in higher survival rate of maize seedlings under heat stress (Li *et al.*, 2013). Exogenous application of salicylic acid reduces the negative effects of abiotic stress in crops and increased plant resistance to heat stress (Hu *et al.*, 2010; Khan *et al.*, 2013). Treating *Triticum aestivum* with 0.5 mM salicylic acid can reduce heat impact by limiting ethylene formation (Khan *et al.*, 2013). Salicylic acid can regulate plant mechanisms in both optimum and stress conditions via cross-talk signalling with other phytohormones (Horvath *et al.*, 2007).

CONCLUSION

This study clearly demonstrates that heat stress affects maize plants growth, and biochemical parameters. It can be overcome by spraying with sodium nitroprusside 50 µM and salicylic acid 75 ppm. It is a simple and easy method to follow by farmers to mitigate the heat stress. We suggest that the farmers of maize could choose nitroprusside 50 μM and salicylic acid 75 ppm for large-scale commercial applications to increase growth, yield and antioxidant activity of maize plant under heat stress.

Conflict of interest

All authors declare that they have no conflicts of interest.

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