

## Induction of Thermotolerant Tomato Plants Using Salicylic Acid and Kinetin Foliar Applications

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**Abstract:** The fruit yield of tomato plant cultivated in summer season where air temperature was exceeded 32°C produce the lowest yield comparing with different growing seasons during the year. This field experiment was carried out to assess the role of foliar applications of kinetin (kin) at 0.05 & 0.1 mM and salicylic acid (SA) at 0.25 & 0.5 mM on ameliorating the deleterious effects of natural heat stress during 2012 and 2013 summer seasons. The both levels of SA significantly increased plant height, plant f.w, branches and leaves no./plant. These positive effects on vegetative growth reflected on enhancing reproductive growth especially for clusters no./plant, fruits no./plant and fruit yield, which directed from the increase in free amino acids and proline. SA at 0.5 mM recorded the lowest value of total phenolics and peroxidase activity while for phenylalanine ammonia-lyase (PAL) was recorded the highest activity. The maximum percentage in fruit set was recorded by kin at 0.1 mM, regardless the lowest value of fruit fresh weight. The highest fruit yield was achieved by SA at 0.5 and 0.25 mM, respectively. Whereas the lowest fruit yield recorded with the application of kin at 0.1 and 0.05 mM, respectively.

**Key words:** Tomato • *Lycopersicon esculentum* • Kinetin • Salicylic acid • Foliar application • Yield • High temperature • Fruit set

### INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown in Egypt. Tomato can grow under a wide range of air temperature however fruit set is limited in a narrow range. Decreasing the percentage of fruit set is correlated with exposure to low or high temperature. The critical factor in tomato fruit setting is the night temperature, the optimal range being 15-20°C [1]. The critical high temperature leading to a decrease in fruit setting is above 32°C while the average minimal temperature is above 21°C [2, 3]. As climate changed with continuous increase in air temperature during summer months, June, July and August, tomato will not be cultivated in the summer months at the year of 2100 due to high temperature which will not be suitable for tomato fruit setting [4]. The most common problem in tomato cultivation is the abscission of flowers due to high

temperatures, where heat stress affect on pollen grain viability, osmotic pressure, fruit setting and fruit yield [5]. The main factor for reducing fruit setting during high temperature is flower metamorphosis, where the stigma in the antheridial cone has higher level [6].

Heat stress induces generation of excessive reactive oxygen species (ROS) resulting in cellular injuries and even collapse of cellular organization. The inhibition in photosynthesis process in the plants grown in high temperature has been attributed to the impairment of structural organization and decrease of photosystem II (PSII) activity [7].

The application of phytohormones is recognized to play an important role in plant response to environmental stresses. Therefore, induction of thermotolerance trait in cultivated high-yielding crops by foliar application with low concentrations of plant growth regulators can be a fruitful effort [8].

The cytokinins play an important role in all aspects of plant growth and development, including cell division, cell enlargement, cell differentiation and organogenesis, nutrient mobilization, chloroplast development, the formation of flowers, fruit set and dry matter formation in plants [9, 10]. Kinetin linked to oxidative processes in the plant cell leading to retarding the senescence in plants, where kinetin has a role as antioxidant protecting DNA against oxidative damage [11]. Kinetin represses abscisic acid effects on plant, where kinetin inhibits the induction of the dormant buds by ABA and alleviates ABA-induced growth inhibition [10]. Therefore, kinetin is known as it has anti-ageing effects in plants [12]. At the membrane level, kinetin inhibits the biosynthesis of jasmonic acid by preventing the formation of ROS or as a direct radical scavenger [13].

Salicylic acid, a common phenolic compound functions as a plant growth regulator and promotes photosynthesis under heat stress by influencing various physiological processes and biochemical reactions [14]. Studies have shown that SA alleviated temperature. SA applied through rooting medium improved photosynthetic capacity of spring wheat [15] and foliar spray of SA alleviated decreases in photosynthesis under salt stress by increasing N assimilation and antioxidant metabolism in mungbean [16]. SA may also influence photosynthesis by interacting with ethylene signaling. The inhibition of ethylene synthesis under heat stress by SA may result in increasing plants response to ethylene and promoting proline metabolism and photosynthesis [7].

Under Egyptian conditions, tomatoes are cultivated through the whole year but the production of tomatoes in the late summer season is limited because of unfavorable high temperature prevailing during flowering and fruit set [17]. So, the objective of this study is alleviating the deleterious effects of high temperature on flowering and fruit yield of tomato plant cultivated in the late summer season using the foliar application of kinetin and salicylic acid.

## **MATERIAL AND METHODS**

A field experiment was conducted during the two growing summer seasons (highest air temperature) of 2012 and 2013 in sandy soil, in a private farm (30°39'N, 32°06'E) at Salehia City, Ismailia Governorate, Egypt, in order to investigate the effect of foliar application with salicylic acid and kinetin on improving growth and productivity of tomato under heat stress.

**Experimental Design, Agricultural Practices and Treatments:** Imported hybrid seeds of tomato, Salyimia 65010 (Technogreen Company) were sown in the shading screen nursery, using foam trays (209 eyes) on 25th of May 2012 and 2013 seasons. Seedlings were transplanted into the field on the first week of July, where the air temperature recorded the highest value during July and August of the summer season (Table 1). Seedlings were spaced 0.5 m apart within the row and 1.6 m between rows. All agricultural praxis management, fertilization, disease and pest control programs were performed as recommended by the Egyptian Ministry of Agriculture and Land reclamation.

The concentrations of foliar application treatments were kinetin (Kin) at 0.05 & 0.1mM and salicylic acid (SA) at 0.25 & 0.5 mM, in addition to distilled water as a control. Plants were sprayed four times with 15-day intervals started at two weeks after transplanting. Tween 20 at 0.1% was used as wetting agent. Treatments were arranged in a complete randomized block design with three replicates.

**Vegetative Growth Characteristics:** Samples of 10 plants were taken at random from each experimental plot at 80 days after transplanting (DAT) to determine plant height, number of branches per plant, number of leaves per plant and plant fresh weight.

**Flowering and Yield Components:** Number of flowers per cluster and clusters number per plant were recorded from beginning of flowering till 70 DAT, where plants grown in this period of time (July and August) exposed to the highest temperature degrees in the year. Five clusters were tagged to determine fruit set percentage which recorded at 70 DAT. Tomato fruits start to harvesting at first of October and after 15 days to determine fruit number per plant and yield fruit fresh weight per plant produced from developed flowers in critical period of high temperature.

**Biochemical Analyses:** All biochemical analyses were determined in the fourth fully expanded leaf of three plants per replicate at 60 DAT.

Total chlorophylls and carotenoids were extracted by grinding 0.2 g leaf tissue with a mortar and pestle using 10 ml N, N dimethyl formamide [18]. The resulting extracts were incubated in the dark fridge overnight. The total chlorophylls and carotenoids concentrations were determined was measured using a spectrophotometer

Table 1: The average of maximum and minimum temperatures in the field during the growing seasons (main of two seasons)

	----- July -----		----- August -----		----- September -----		October
Air temperature	1-15	16-31	1-15	16-31	1-15	16-30	1-15
Av. max	38.7	39.4	40.0	39.3	38.8	36.3	33.2
Av. min	20	19.5	19.8	19.4	18.5	16.6	14.9

(Mapada UV 1200) at 470, 647 and 664 nm. Concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophylls and carotenoids were calculated using formulae described by Lichtenthaler and Wellburn, [19].

Total soluble sugars were extracted from 0.5 g leaf tissues by 80% hot ethanol as described by A.O.A.C. [20] and determined using phenol-sulfuric acid method described by Dubois *et al.* [21]. Total free amino acids were determined according to the method described by Swamy [22]. The pink color developed with ninhydrin reagent was measured using a spectrophotometer (Mapada UV 1200) at 570 nm.

Proline concentration was determined according to the method of Troll and Lindsley [23]. Fresh leaf samples (0.5 g) were ground and homogenized with one volume of 100 mM sodium phosphate buffer (pH 6.0). The samples were centrifuged for 10 min at 16.000 x g. The reaction mixture contained 200 µl of the supernatant and 1 ml of ninhydrin solution (2.5 g dissolved in 100 ml of ortho-phosphoric acid, acetic acid and water 15: 60: 25. V: V: V). The reaction proceeded for 1 h in boiling water bath and the developed dye was extracted with 1 ml of toluene and measured by the spectrophotometer at 515 nm using a spectrophotometer (Mapada UV 1200). The proline concentration was determined by the standard curve of L-proline.

Total Phenolic concentration was determined using Folin-Ciocalteu method described by Singleton and Rossi [24] with catechol as standard, which read at 650 nm.

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) concentration by thiobarbituric acid (TBA) reaction as described by Heath and Packer [25]. One gram of fresh leaves tissue was homogenized in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 x g for 5 min then 4 ml of TBA solution (0.5 g TBA / 100 ml TCA 20%) was added to 1 ml of the supernatant, the mixture was heated to 95 °C for 30 min and then quickly cooled in ice bath. The contents were centrifuged at 10000 x g for 15 min and the absorbance of suspension was measured at 532 nm in spectrophotometer (Mapada UV 1200). The concentration of MDA was calculated using an extinction coefficient of 155 mM cm<sup>-1</sup> and was expressed as µmol g<sup>-1</sup> f.w.

**Determination of Antioxidant Enzymes Activity:** Half gram of leaf tissue was homogenized in 4 ml of 100 mM chilled sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone (w/v) at 4°C. The homogenate was centrifuged at 11.000 x g for 15 min at 4 °C. Supernatant was used to measure the activities of phenylalanine ammonia-lyase (PAL) and peroxidase (POD). Protein concentration was determined according to the method of Bradford [26].

PAL (EC: 4.3.1.5) activity was determined by Brueske method [27]. The PAL assay reaction mixture consisted of 100 µl crude enzyme extract and 900 µl of 6 µmol L-phenylalanine in 500 mM Tris-HCl buffer (pH 8.5). The mixture was incubated at 37°C for 1 h. The absorbance was measured by spectrophotometer at 290 nm. One unit was determined as 1 iM of L-phenylalanine converted to trans-cinnamate and NH<sub>3</sub> min<sup>-1</sup>. The produced amount of trans-cinnamic acid was calculated by ( $\epsilon = 9630 \text{ M}^{-1} \text{ cm}^{-1}$ ).

POD (EC: 1.11.1.7) activity was assayed by the method of Maehly and Chance [28]. The reaction mixture (2.9 ml) consisted of 0.25 % (v/v) guaiacol in 10 mM sodium phosphate buffer (pH 6 containing 10 mM H<sub>2</sub>O<sub>2</sub>). Volume of 100 µl of the crude enzyme extract was added to initiate the reaction which was measured at 470 nm per min. One international (IU) of enzyme activity was expressed as  $\Delta \text{OD} = 0.01$  POD activity expressed as unit min<sup>-1</sup> mg<sup>-1</sup> protein.

**Statistical Analysis:** Data of the two seasons were arranged and statistically analyzed using CoStat software (version 6.4, CoHort Software, USA) according to the method described by Gomez and Gomez [29]. One-way ANOVA was used to test for significant differences among foliar application substances. A post hoc Tukey's HSD test was used to test for significant differences between treatments means.

## RESULTS AND DISCUSSION

**Vegetative Growth Characteristics:** All environmental stresses including high temperature stress have a deleterious effect on plant growth and yield components. The protective effects of treatment with

kinetin on vegetative parameters are illustrated in Table 2. The lowest level of kin (0.05 mM) has a significant increase over control in plant fresh weight, which refer mainly to its effect on increasing plant height, branches no. and leaves no./plant. While kin at 0.1 mM decrease the plant fresh weight comparing to control, which refer mainly to decreasing plant height, branch diameter and leaf area (non-published data). This decrease in branch diameter under kin at 0.1 mM refer to the branches formed later than other treatments. These results are in good agreement with those finding by Mukherjee and Kumar [30], where branching number, leaf number and leaf area of *Cajanus cajan* was significantly higher in Kin 0.05 mM, in addition to a significant increase in DNA, RNA and protein contents during maturity and senescent phase. Cytokinins are active in the maintenance of ongoing processes and nutrient mobilization in the shoot, thus they inhibit shedding by stimulating auxin production [11]. Kinetin linked to oxidative processes in the plant cell leading to retarding the senescence in plants. The specific response of RNA polymerase I transcription to kinetin suggests that cytokinins may act as general regulators of protein synthetic capacity and growth status in plant cells. It has been shown that the incubation of detached tomato leaves with kinetin activates the endogenous cytokinin pathway for the delay of senescence by minimizing contents of ABA, ethylene or other growth retardants, induces the expression of endogenous cytokinin-inducible extracellular invertase [11].

The highest values in all vegetative parameters were recorded with both salicylic acid tested levels (0.25 and 0.5 mM). The best application was for SA at 0.5 mM, where the highest value of plant fresh weight could refer mainly to the increase in plant height and leaves no./plant (Table 2). Salicylic acid plays exclusive role in plant growth, development and ions uptake. It affects ethylene biosynthesis and reverses the effects of ABA on leaf abscission [14]. Moreover, the decrease of net photosynthetic rate under heat stress was alleviated by SA, through maintaining a higher Rubisco activation state and greater PSII efficiency [31]. SA also accelerated the more rapid recovery of PSII function after heat stress and may be related to higher levels of heat shock protein (HSP21), which reflected on an increase in net photosynthetic rate [31].

**Flowering and Yield Components:** Though the preferred kinetin for enhancing vegetative growth was for kin at

0.05 mM (Table 2), the highest values in flowers no./cluster, fruit set percentage and fruits no./plant recorded with kin at 0.1 mM (Table 3). Fruit set % did not significantly affect under all tested treatments as compare to control (52.4% in 1<sup>st</sup> season) except for kin at 0.1 mM, which recorded the highest significant value (65.7 % in 1<sup>st</sup> season). This increase in fruit set % led to an increase in fruits no./plant (42 in 1<sup>st</sup> season) as compared with control (32.3 in 1<sup>st</sup> season). Although, the highest significant value in fruit set % recorded with kin at 0.1 mM, the highest significant value in fruits no./plant recorded with SA at 0.25 mM. This increase in fruit no./plant under SA treatments, refer mainly to the positive effect of SA on increasing the clusters no./plant, or with another words, SA protected and enhanced the development of reproductive growth of tomato plants under high temperature. These positive effects of SA on fruits no./plant extended to fruit fresh weight and fruit yield/plant, where the maximum yield value recorded with SA at 0.5 mM, which refer mainly to the increase in fruit fresh weight (Table 3), which may be directed from the increase in leaves no./plant and plant fresh weight (Table 2). In this connection, Kaushal *et al.* [32] mentioned that SA has an important role in many physiological process including translocation of solutes, photosynthesis, induction of flowering and protein synthesis, which may be reflected in increasing clusters no./plant in this study (Table 3).

In contrast to the positive effects of kinetin on flowering parameters, the average fruit fresh weight was decreased significantly especially for kin at 0.1 mM which recorded 34.8 g/fruit as compared with 52.9 g/fruit in control (Table 3). This decrease in fruit yield under kinetin treatments may be refer to reducing the translocation of assimilates from leaves to fruit, where assimilates directed first to the new growths of branches and leaves formed latterly (non-published data), which reflecting on fruit size and weight. However, kinetin application several times promotes cell division in both vegetative and generative growth with increasing sink capacity leading to increase internal competition between vegetative and generative organs. Since, kinetin is known as it has anti-ageing effects in plants [12]. It represses the effects of abscisic acid on plant, leading to retarding the senescence in plants, which reflected on enhancing vegetative growth more than reproductive growth. Salicylic acid plays exclusive role in thermogenesis and flower induction [14].

Table 2: Influence of kinetin (kin) and salicylic acid (SA) as foliar application on vegetative growth parameters of tomato plant in both summer seasons (2012 and 2013)

Foliar treatments (mM)	1st season				2nd season			
	Plant height (cm)	Branches (no./plant)	Leaf (no./plant)	Plant f.w. (g)	Plant height (cm)	Branches (no./plant)	Leaf (no./plant)	Plant f.w. (g)
Control	76.0 bc	10.7 a	214.3 b	947 b	82.3 b	11.3 a	251.3 b	1381.7 c
Kin 0.05	80.7 ab	11.7 a	235.7 ab	1423 a	83.0 b	12.0 a	260.7 b	1581.7 b
Kin 0.1	74.3 c	11.3 a	238.7 ab	847 b	80.0 b	11.7 a	251.7 b	1188.3 d
SA 0.25	85.0 a	11.7 a	243.7 ab	1330 a	88.7 a	12.0 a	260.0 b	1791.7 a
SA 0.5	85.7 a	11.3 a	256.7 a	1392 a	92.0 a	12.0 a	292.7 a	1806.7 a

Means followed by different letters are significantly different at P< 0.05 level; Tukey's HSD test. Where f.w. = fresh weight.

Table 3: Influence of kinetin (kin) and salicylic acid (SA) as foliar application on reproductive parameters and yield components of tomato plant in both summer seasons (2012 and 2013).

Foliar treatments (mM)	Clusters (no./plant)	Flowers (no./cluster)	Fruit set %	Fruits (no./plant)		Average fruit f.w. (g)	Fruits yield (g f.w./plant)
				1st season	2nd season		
Control	17.1 c	3.6 bc	52.4 b	32.3 c	37 c	52.9 b	1706 b
Kin 0.05	16.5 c	3.8 ab	55.3 b	35.0 c	38 c	45.2 c	1582 b
Kin 0.1	16.4 c	3.9 a	65.7 a	42.0 b	48 ab	34.8 d	1457 b
SA 0.25	25.2 a	3.5 c	54.4 b	47.7 a	55 a	49.0 bc	2331 a
SA 0.5	21.3 b	3.7 abc	53.4 b	42.0 b	42 bc	59.1 a	2478 a
Control	16.6 bc	4.2 a	53.2 b	37 c	37 c	58.2 ab	2153 b
Kin 0.05	14.6 c	4.4 a	59.4 ab	38 c	38 c	53.8 b	2045 b
Kin 0.1	16.3 bc	4.5 a	65.9 a	48 ab	48 ab	39.5 c	1890 b
SA 0.25	22.3 a	4.4 a	55.5 b	55 a	55 a	51.1 bc	2800 a
SA 0.5	18.7 b	4.3 a	53.2 b	42 bc	42 bc	67.4 a	2848 a

Means followed by different letters are significantly different at P< 0.05 level; Tukey's HSD test. Where f.w. = fresh weight

**Biochemical Content Changes:** Data presented in Table 4 show that, there are no significant differences in photosynthetic pigments of tomato plant between most treatments comparing with control. The highest values in all photosynthetic pigments forms were for SA at 0.25 and 0.5 mM and kinetin at 0.05 mM. In this regard Kaushal *et al.* [33] mentioned that heat stress above 32°C decreased chlorophyll concentration and chl *a/b* ratio in tomato plants. Khan *et al.* [7] reported that lower concentration of SA improves photosynthetic characteristics, while higher SA concentration has been found to disintegration of plastid structure and increased accumulation of NADPH by limiting the Calvin cycle. Kinetin applications tended to slow down the rate of chlorophyll loss under heat stress [33]. Mukherjee and Kumar [30] found that kinetin was effective in enhancing the chlorophylls concentration, the most effective was Kin 0.05 mM. Kinetin application control the chlorophylls breakdown in leaves at various stages including senescence.

The highest significant value of total soluble proteins was for kinetin application at 0.1 mM (4.9 mg g<sup>-1</sup> f.w in 1<sup>st</sup> season) followed by kin at 0.05 mM (4 mg g<sup>-1</sup> f.w in 1<sup>st</sup> season), which reversed parallel with that the minimum values in total free amino acids were for kinetin at 0.1 mM (0.96 mg g<sup>-1</sup> f.w in 1<sup>st</sup> season) and 0.05 mM (1.02 mg g<sup>-1</sup> f.w in 1<sup>st</sup> season) respectively (Table 5). Not only kin at 0.1 mM led to a reduction in free amino acids compared with other treatments, but also recorded the lowest value in total soluble sugars (4.5 mg g<sup>-1</sup> f.w in 1<sup>st</sup> season). So, kinetin application led to a reduction in photo-assimilates

in leaves of tomato plant and an increase total soluble proteins, which suggest that most of photo-assimilates in leaves used also in leaves as a sink more than fruits (Table 5). This hypothesis supported by that the lowest fruit fresh weight were for kinetin applications (Table 3), while these levels of kinetin led to an increase in branches no./plant and leaves no./plant (Table 2). Kinetin application significantly slowed down the protein breakdown under stress conditions [30]. Salicylic acid treatment recorded the highest significant values in proline concentration, which suggest that application of salicylic acid gave more tolerant plants to high temperature than other treatments (Table 5). Although, SA at both levels did not increase soluble proteins in leaves to reach a significant level comparing to control, the total free amino acids were increased in leaves subjected to treatment of SA at 0.25 mM. In this regard, Khan *et al.* [7] reported that SA treatment (0.5 mM) alleviated heat stress by increasing proline production. Proline accumulation is one of the adaptive mechanisms leading to plant survival under stress. It regulates abiotic stress by detoxifying excess ROS, cellular osmotic adjustment, protection of biological membranes and stabilization of enzymes/proteins. Under environmental stress, proline acts as an antioxidant by utilizing NADPH for the reduction of glutamate to pyrroline-5-carboxylate (P5C) -which act as an intermediate product of both proline biosynthesis and catabolism- and further from P5C to proline and generates NADP<sup>+</sup> which used as an electron acceptor and for the restoration of the Calvin cycle under stress conditions [7].

Table 4: Influence of kinetin (kin) and salicylic acid (SA) as foliar application on photosynthetic pigments of tomato plant in both summer seasons (2012 and 2013).

Foliar treatments (mM)	1st season				2nd season			
	Chl a mg/g f.w.	Chl b mg/g f.w.	Chl a+b mg/g f.w.	Carot mg/g f.w.	Chl a mg/g f.w.	Chl b mg/g f.w.	Chl a+b mg/g f.w.	Carot mg/g f.w.
Control	0.63 b	0.53 a	1.15 a	0.32 a	0.62 b	0.54 ab	1.17 b	0.32 b
Kin 0.05	1.05 ab	0.43 a	1.47 a	0.53 a	1.03 a	0.39 b	1.41 ab	0.52 a
Kin 0.1	0.68 ab	0.53 a	1.21 a	0.39 a	0.62 b	0.41 b	1.04 b	0.36 b
SA 0.25	1.08 a	0.84 a	1.93 a	0.57 a	0.99 a	0.75 a	1.74 a	0.53 a
SA 0.5	0.82 ab	0.65 a	1.47 a	0.45 a	0.81 ab	0.63 ab	1.44 ab	0.45 ab

Means followed by different letters are significantly different at P< 0.05 level; Tukey's HSD test. Where f.w. = fresh weight, Chl = chlorophyll, Carot = carotenoids.

Table 5: Influence of kinetin (kin) and salicylic acid (SA) as foliar application on total free amino acids (AA), proline, total soluble proteins (TS Proteins) and total soluble sugars (TSS) concentrations of tomato plant in both summer seasons (2012 and 2013)

Foliar treatments (mM)	1st season				2nd season			
	AA (mg g <sup>-1</sup> f.w.)	Proline (µg <sup>-1</sup> f.w.)	TS Proteins (mg g <sup>-1</sup> f.w.)	TSS (mg g <sup>-1</sup> f.w.)	AA (mg g <sup>-1</sup> f.w.)	Proline (µg <sup>-1</sup> f.w.)	TS Proteins (mg g <sup>-1</sup> f.w.)	TSS (mg g <sup>-1</sup> f.w.)
Control	1.41 b	3.0 b	3.6 b	6.1 a	2.01 b	3.3 b	4.9 b	7.9 a
Kin 0.05	1.02 c	3.0 b	4.0 ab	5.2 ab	1.34 c	3.6 b	5.5 b	6.6 ab
Kin 0.1	0.96 c	3.6 ab	4.9 a	4.5 b	1.46 c	4.5 a	6.5 a	5.7 b
SA 0.25	1.79 a	3.9 a	3.9 ab	5.3 ab	2.34 a	4.5 a	5.4 b	6.7 ab
SA 0.5	1.37 b	4.1 a	3.9 ab	5.1 ab	2.13 b	4.5 a	5.4 b	6.1 b

Means followed by different letters are significantly different at P< 0.05 level; Tukey's HSD test. Where f.w. = fresh weight

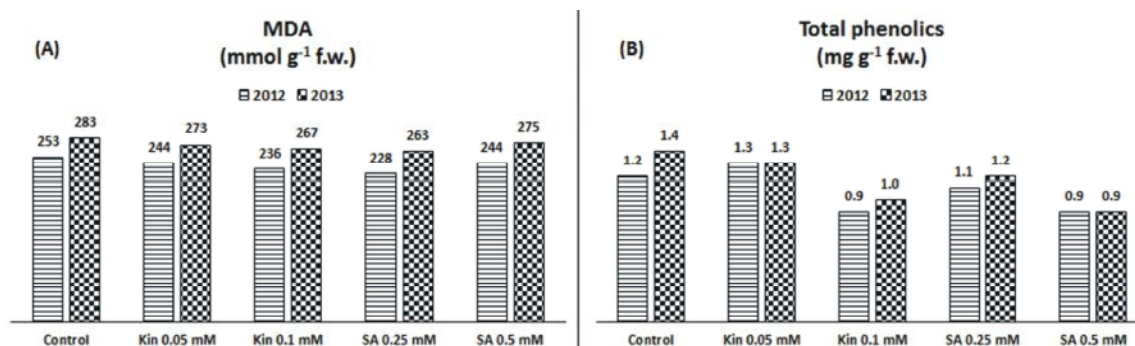


Fig. 1: Influence of kinetin (kin) and salicylic acid (SA) as foliar application on MDA and total phenolics in leaves of tomato plant at 60 DAT in both summer seasons (2012 and 2013)

Malondialdehyde (MDA) concentration indicate to the level of lipid peroxidation and integrity of membrane lipids. Although, there is no significant differences between all tested treatments in MDA levels, all tested levels of kinetin and salicylic acids reduced the amount of MDA in leaves, which indicate to the role of these treatments in protecting the membrane lipids from oxidation (Figure 1:A). The concentration of total phenolics decreased to a significant level with the higher level of both kinetin and salicylic acid (Figure 1:B). This decrease in the concentration of total phenolics indicated that the oxidant substances in treated plants was decreased. Since phenolics have multiple roles in plants, as structural components of cell walls, exhibit a prominent contribution in growth and development as well as improving tolerance of plants towards abiotic stresses where act as sources of electrons and protons for reactive oxygen species [34]. So, decreasing phenolics concentration under SA treatment may be refer to that the stress status of tomato plants is in low level.

Salicylic acid at 0.25 mM recorded the maximum significant level in peroxidase activity, followed by kinetin

treatment at 0.1 mM (Figure 2:A). The lowest significant level in peroxidase was achieved by SA at 0.5 mM, which suggest that under the higher level of SA treatment, the amount of H<sub>2</sub>O<sub>2</sub> in leaves tend to decrease below control, which could be refer to the increase in the activity of superoxide dismutase and catalase enzymes. While increase the activity level of POD under SA at 0.25 mM indicate to the activity of superoxide dismutase and catalase enzymes are not sufficient to catalyze H<sub>2</sub>O<sub>2</sub> concentration, which reflecting on increasing POD activity. Phenylalanine ammonia-lyase (PAL) recorded the highest significant values under all tested levels of salicylic acid (Figure 2:B). These results indicated that under high temperature stress, the enhanced activities of PAL a key enzyme at the gateway of propanoid biosynthetic pathway could activate the cellular antioxidant enzymes, which could scavenge the excess ROS and maintain the cellular redox status and thereby reduce the photo- and oxidative damages caused by high temperature stress, as been recorded under salinity stress in maize plant [35].

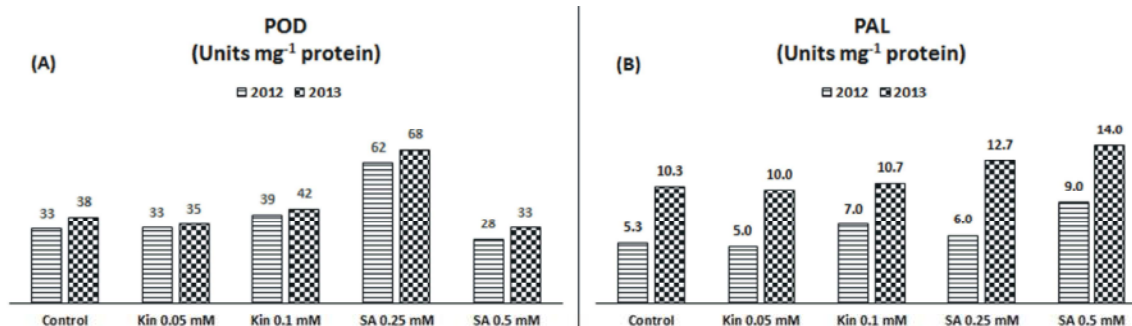


Fig. 2: Influence of kinetin (kin) and salicylic acid (SA) as foliar application on enzymatic activities of POD and PAL in leaves of tomato plant at 60 DAT in both summer seasons (2012 and 2013).

Heat stress causes multifarious and often adverse, alterations in plant growth, development, physiological processes and yield (Tables 1 & 2). One of the major its consequences is the excess generation of reactive oxygen species, which leads to oxidative stress. Plants is able, in some extent, to tolerate heat stress by physical changes within the plant body and frequently by creating signals for changing metabolism. Plants alter their metabolism in various ways in response to heat stress, particularly by producing compatible solutes that are able to organize proteins and cellular structures, maintain cell turgor by osmotic adjustment and modify the antioxidant system to re-establish the cellular redox balance and homeostasis [36]. In tobacco cell culture exogenously applied kinetin induced the activity of PAL enzyme [37].

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