Comparison among different amelioratives on improvement of tomato growth, yield and quality under low temperature condition

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Abstract

Salicylic acid (SA), acetyl salicylic acid (ASA), melatonin (Me), potassium silicate (PS) and potassium phosphonate (PP) are known to modulate physiological processes and lessen the effects of abiotic stress. High and low temperature are the most harmful sort of abiotic stress, limiting plant survival and productivity. This study aims to compare between the efficiency of the foliar application of SA (1 mM), ASA (178 mM), Me (100 µM), PS (250 mg/L), PP (3 m/L), and mix between ASA and PS (Mix), comparing with an untreated control, on the morphology, yield, and quality-related parameters of tomato plants stressed at low temperatures under Egyptian conditions. The treatments of Mix, ASA and SA increased all antioxidant Enzymatic (CAT, POD and PPO) activities. PS increased the activity of POD and PPO, while Me indued PPO only. There was improvement for non- Enzymatic compounds, namely total free amino acid, by Mix and Me, total indoles, by Mix, SA and PP and total phenols by PS, while the treatments reduced the concentrations of proline and total sugars in plants. Mix, ASA and Me improved chlorophyll B and carotenoids. Mix and SA have a positive effect on preserving tomato plants, yield, and fruit quality, Tomato plants treated with Mix showed positive effects on all traits of morphological characters, yield, fruit quality, and were significantly superior than using each of ASA and PS alone. So, the Mix treatment between ASA and PS is considered very effective as a foliar spray meliorative for tomato plants under low temperature condition.

Keywords: Tomato (*Solanum lycopersicum* L.), foliar application, low temperature injury, yield, fruit quality, antioxidant.

1. Introduction

Low and high temperatures are by far the most important environmental stressors that affect crop production [1]. Low temperature (LT) is one of the main environmental factors regulating the geographic range of plants and crop production [2]. LT stress is one of the most limiting ecological variables for horticultural yields particularly vegetables, which accounts for significant crop losses [3]. LT affects the way that plants interact with water because it reduces hydraulic conductivity and stomatal control [4]. It impairs plant development and survival by decreasing the amount of water available to the plant, decreasing membrane fluidity, and creating an imbalance between the amount of light energy absorbed by photosystems and the amount of energy required by metabolic processes [5]. Moreover, LT also reduces the capacity and efficiency of photosynthesis through changes in pigment composition, a reduction in chlorophyll fluorescence, and decreased chloroplast growth [6].

Chilling stress may create necrotic lesions on leaves, impede leaf development, extend cell cycle with decreased cell formation, induce wilting, and increase susceptibility to pathogens and diseases [7]. Root growth is impeded by chilling stress because root length and biomass are reduced [8], which decreases the root system's volume in order to absorb nutrients and water [9]. In later phases of plant growth and development, chilling stress causes significantly slowed growth that either limits or prevents the production of flowers and fruits [10].

Vegetables have a significant spot in the broadening of horticulture and have assumed an essential part in food and dietary security of consistently developing populace of Egypt. Tomato (*Solanum lycopersicum* L.) is one among the most popular and cultivated horticultural species in the global [11]. Egypt is fifth in the world for production, reaching 6,624,733 tonnes in 2018 from 385,004.8 feddens at an average of 17.21 tons/fed [12]. Tomato is an abundant source of vitamins, minerals, antioxidants and dietary fibers [13] that reduces the prevalence of chronic diseases such as anti-aging, anti-cancer, and safeguarding against obesity, diabetes mellitus, cardiovascular disease, and neurodegenerative illnesses [14]. Tomato plants are susceptible to chilling temperatures (0-15°C) throughout their growth. Chilling stress can result in wilting, necrotic lesions on leaves, delayed leaf development, prolonged cell cycles with reduced cell output, and increased susceptibility to pathogens [7].

Salicylic acid (SA) is a naturally occurring phenolic molecule that serves as a signaling plant growth regulator (phytohormone), improving plant tolerance to a variety of abiotic conditions such salinity, drought, and severe temperatures [15]. It has a variety of functions in physiological processes like seed germination and plant growth [16], vegetative growth [17], nutrient uptake and transport [18], and photosynthetic rate, stomatal regulation, and transpiration [17,19]. It is well known how SA helps various crops cope with drought stress [16]. For instance, it has been documented that the exogenous application of SA in low concentration under drought stress considerably benefited the development and physiology of tomato and bean (*Phaseolus vulgaris* L.) [15,16]. SA plays important roles in defense responses as signaling molecules [20]. SA has just been introduced to the list of well-known classical plant hormones and has demonstrated potential as a technique for improving plants' tolerance to abiotic stress [21].

Phenolic compounds such as Acetyl salicylic acid (ASA) when used exogenously, have been found to increase plants ability to cold stress [22]. The application of phenolic compounds helps improve stress tolerance in plants. However, the ideal ratio of phenolic compounds to ease tomatoes' low-temperature stress is not well understood. Additionally, there are no field assessments of phenolic substances used exogenously [1].

Melatonin (Me) is a tryptophan-derived substance (N-acetyl-5-methoxytryptamine) mediates a variety of biological activities in both humans and animals [23]. Its role as a signaling molecule during biotic and abiotic stress was also recently discovered [24].

Atlante is a complements fertilization (phosphorus 30%, potassium 20%) as a potassium phosphonate (PP). According to [25] spraying plants with PP during salt stress reduced antioxidant and monoaldehyde (MDA) production, while promoting more growth in salt-tolerant genotypes than salt-sensitive genotypes. The use of potassium under salt stress increased tomato plant growth and decreased MDA levels, indicating that potassium is an efficient ameliorating agent against salt-induced oxidative damage.

Potassium silicate (PS) has the ability to relieve salt stress due to its high effectiveness in raising relative water content, the membrane stability index, antioxidant activity like peroxidase and catalase, and the reduction of proline concentration. Therefore, foliar application in the form of PS can be used as a promising alternative to mitigate abiotic stress of water deficit in tomato [26] and stress caused by low temperatures and salt in immature mango trees [27]. However, there is still a lack of information about how PS helps tomatoes that have been damaged by low temperatures.

This study aimed to compare among some ameliorative compounds, namely acetylsalicylic acid, potassium silicate, melatonin, potassium phosphonate, salicylic acid, and a mixture between acetylsalicylic acid and potassium silicate on morphology, yield, and quality related traits of tomato plants at low temperature stress.

2. Materials and Methods

a. The experimental Conditions

Two field experiments were carried out at Al Mansoureyah village, Giza governorate (30° 12′ E; 31° 08′ N) Egypt, during the two winter growing seasons of 2019 and 2020. The average minimum, maximum temperatures and Relative humidity of both years are shown in (Figure 1).

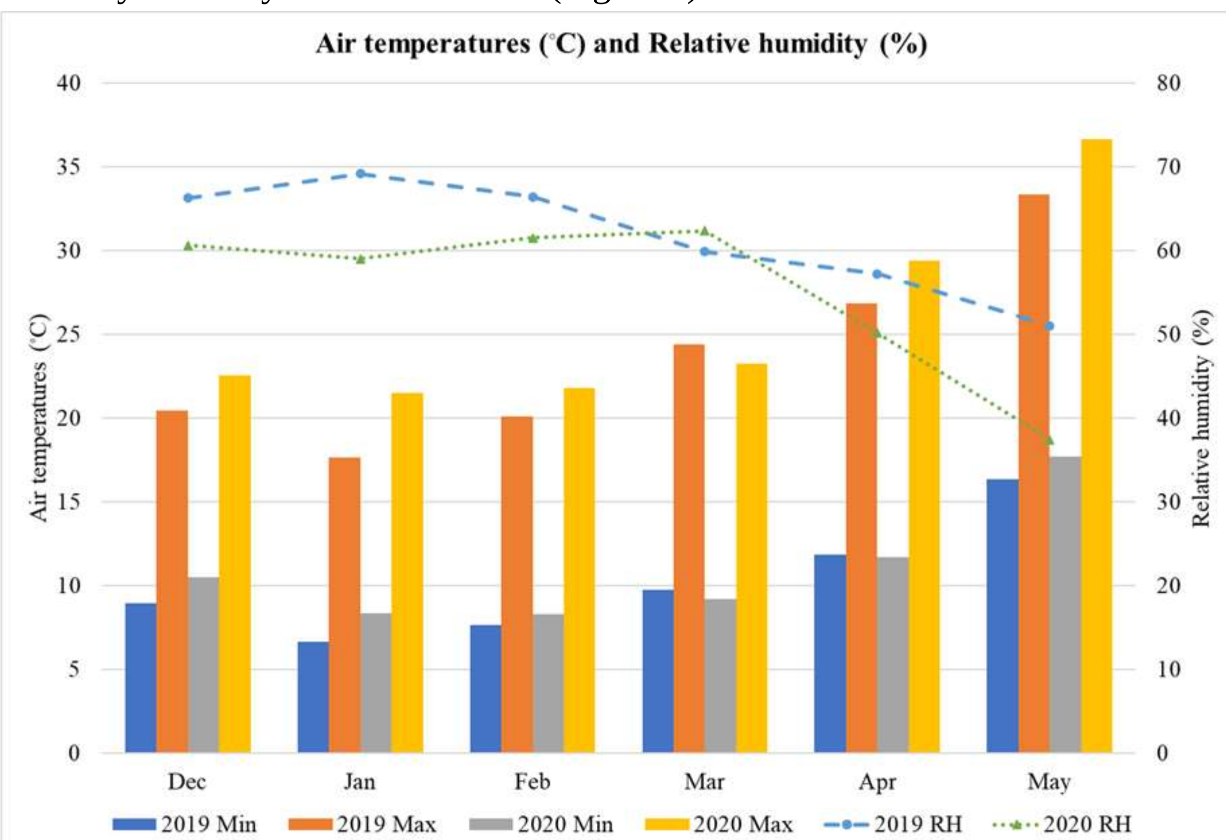

Fig. 1. The average minimum, maximum temperatures (°C) and Relative humidity (%) during 2018-19 and 2019-20.

The experiment was designed as randomized complete block design (RCBD) with three replications per treatment. Each plot measured 14 m^2 with two rows of 7 m length and 1 m width (26 plants per plot). The treatments were applied as foliar spray three times, 30, 45 and 60 days after transplanting. The experimental setup consisted of 8 treatments as shown in (Table 1). Cultural management was applied according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation.

Table 1. List of the product recommended dose and sources

	The product	**The recommended dose**	**Source**
1	Control	water	Irrigation source
2	Acetyl salicylic acid Aspirin (ASA)	178 mM (1cap/10L)	The Arab drug Co.
3	Potassium silicate (PS)	250 mg/L	Peptech Biosciences Ltd Co.
4	The mix between (ASA and PS)	178 mM + 250 mg/L	**-**
5	Salicylic acid (SA)	1mM/L	Al-Alamia for Chemical Co.
6	Melatonin (Me)	100µM/L	Puritan's Pride Co.
7	Atlante (Atl) (phosphorus 30%, potassium 20%) potassium phosphonate	3m/L	Shoura Chemicals Co.
8	Tunnel*****	polyethylene	Hyma-Plastic Co.
9	Carbine 5SL******	60cm/100L	Phytorgan S.A. Co.

***Tunnel plants protected by shelterbelt (Covered by polyethylene, as per the recommendation of the Egyptian Ministry of Agriculture and Land Reclamation), **Carbine PCPA (4-Chlorophenoxyacetic acid) 5% SL.**

b. Plant Material

Tomato (*Solanum lycopersicon* L.) cultivar Platinium (Nunhems Seed Com. Netherlands), was used. This cultivar is a very conventional and well-known for its production in winter climates.

Seeds were sown in nursery foam trays (209 eyes) containing mixture of cocopeat and vermiculite (1:1). The trays were kept in a plastic greenhouse to protect the transplants from the low temperature. Thirty days after sowing, transplants were planted in open field. Tomato plants were transplanted on 15st December 2019 and 2020, seasons under open field conditions, at spaces 1 m between rows and 50 cm between plants inside the rows.

c. Data Recorded

(1) Vegetative Growth Characters

Thirty days after transplanting the low temperature injury index was recorded. It was classified into the following six points: 0 for non-low temperature injury; 1 for 0-20%; 2 for 21-40%; 3 for 41-60%; 4 for 61-80%; and 5 for 81-100% of leaf area damage. for 60 days take 50% flowering according to [28].

Three months after transplanting, five plants from each plot were randomly harvested and the following characteristics were recorded.

Plant height (cm), measured from the ground level to the apical meristem of the main stem. Number of branches, determined by counting all branches of the plant. Stem diameter (cm), measured by Calipers. Number of leaves per plant, determined by counting all leaves of the plant including new tips and sprouts. Plant leaf area (cm), average leaf area of the fifth leaf from the top was measured by portable leaf area meter (Leaf area per plant was calculated by using ImageJ2 program software for multidimensional image processing and analysis version 0.33.0.). Plant fresh weight (kg/plant), was mathematically obtained by summing leaf and stem fresh weight. Fruit set percentage, the percent ratio between the number of flowers and fruits of the fifth cluster at harvest. Early was determined as the yield of the first picking (Ton/Fed). At the end of the crop cycle, the total production of fruits throughout the harvesting period was calculated as a total yield. The total yield for each treatment was calculated by weighing the fruits picked from all plants in each plot and converting the weight into ton per fed.

(2) Chemical analysis

Leaves

Leaf pigments contents, namely, chlorophyll a, b and carotenoid (mg g^{-1} fresh weight) were spectrophotometrically determined and calculated as described by according to [29]. Total indoles, were determined using P-dimethyl amino benzaldehyde (PDAB) test as described by [30]. Total free amino acids and total phenols, They were determined according to [31]. Total sugars, Determination of total soluble sugar was performed according to [32]. Proline content, was determined using the Bates's method [33]. Enzyme's activity of Catalase (CAT), Peroxidase (POD) and Polyphenol oxidase (PPO) was determined according to [34] by using spectrophotometer model UV-Vis spectronic 601.

Fruits

Lycopene content, was measured as described by [35]. β-Carotene content, was measured as described by [36]. Total soluble solids % (TSS), values were measured in fruit juice at room temperature by using a hand refractometer (Atago digital, Japan) in three different readings for each replicate according to the method by the [37]. Total acidity (%), was measured as mentioned in [37], the fruit juice was titrated with sodium hydroxide solution (0.1 N) in the presence of few drops from phenol naphthalene as indicator. Results were expressed as (g) anhydrous malic acid per 100g. Vitamin C (mg/100g FW), was determined as described [37]. Results were expressed as mg ascorbic acid/100 g FW.

d. Statistical analysis.

Data for current study were statistically analysed using Mstat-C and significant differences were calculated using Duncan multiple range test at p = 5% level.

3. Rustles and discussions.

a. Low temperature injury (LT injury).

The effects of cold stress, which includes chilling of less than 15 degrees Celsius and freezing temperatures below zero, have a drastically impact on plant development and growth [38]. LT affects plants at all stages of development, from germination through maturity [39]. Reduced seedling growth is one of the typical LT injury in plants that leads to stunting, production of white specks, white bands or leaf yellowing and whitening, and under extreme condition withering after transplanting and subsequent seedling death [40]. On the other hand, Low temperature causes disruption of meiosis during the development of male female gametophytes, pollen sterility, pollen tube deformation. reduced stigma receptivity, as well as a delay to the fertilization process, while during flowering LT induces bud abscission [41].

Our results revealed that in nontreated plants (control) revealed the most serious symptoms of a chilling damage, including wilting, necrosis, and then desiccation of the majority of the leaves (Figure 2). On the contrary, all treatments were able to reduce low temperature injury, by reducing the leaf area damaged at low temperatures. The lowest values of the temperature injury index were recorded in the Mix treatment between ASA and PS. This treatment was superior than using each substance alone.

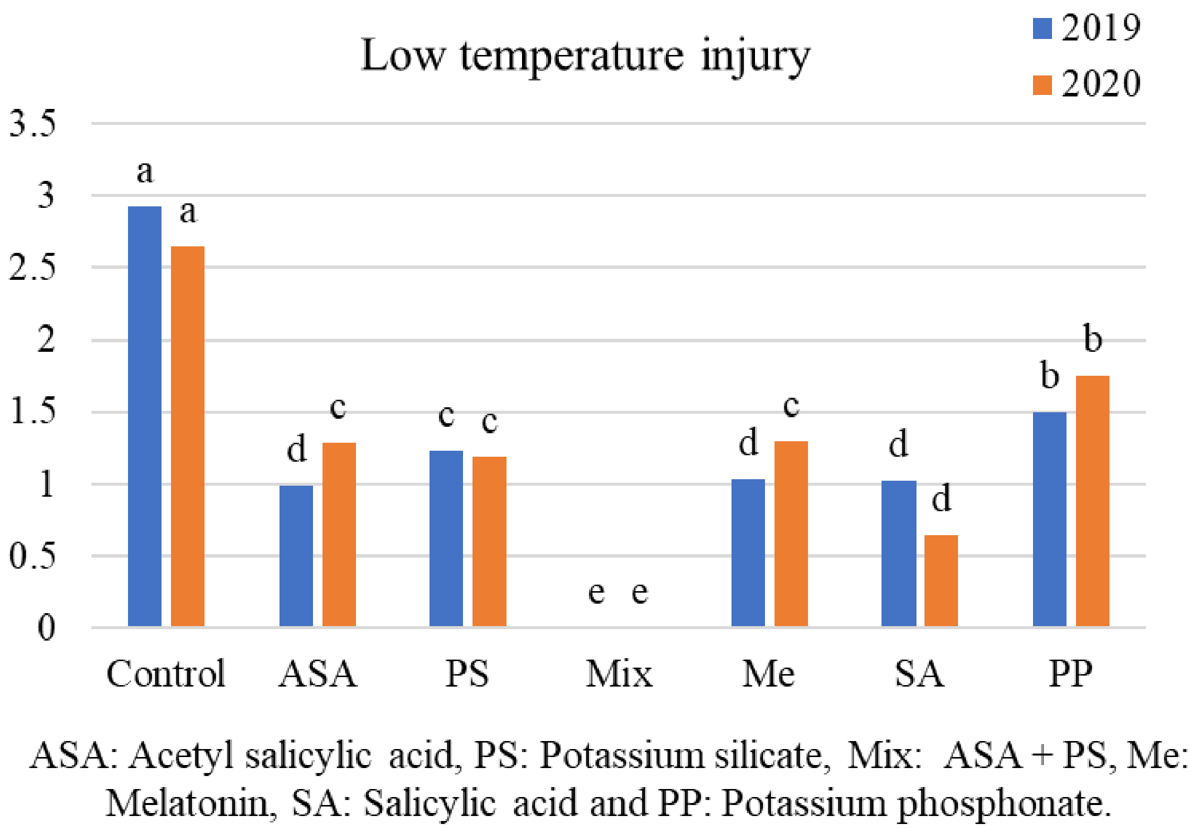

Figure 2. Low temperature injury index on tomato plants with foliar applications treatments.

b. Morphological Characterization.

The present study has observed that low temperature stress decreased plant development, having negative and inconsistent impacts on biomass accumulation. Similarly, [42] observed that low temperature stress caused a considerable reduction in fresh and dry mass of root, shoot and root length and shoot in watermelon.

Our results showed that cold alleviating compounds had significant influence in reversing the growth inhibition. All treatments stimulated the growth characteristics of tomato plants under LT conditions. Mix treatment was proved to be the best treatment in maintaining good vegetative growth, and followed by SA, PS and ASA (Table 2). On the contrary, PP showed the lowest values in all vegetative growth charters (plant height, number of branches, number of leaves, leaf area and fresh weight), except stem diameter, which was higher than all other chemical treatments. In a previous study, [43] recorded imrovement in shoot fresh mass of 40 old maize plants, subjected to 2 days chilling due to spraying the plants with 10 mM Si 10 days before chilling. On the other hand, [28] found that the effect of application of silicate fertilizer on the growth of tomato seedling under low temperature (below 7, 10^{o}C during night time or daily mean air temperature was 18^{o}C) dependet on silicate level. While silicate fertilizer at 16 mM regisered improvement in tomato seedling quality (No. of leaves , leaf area and fresh weight) the application of silicate fertilizer at 256 mM shoed a reverse effect.

Previous studies indicated that ASA at 0.5 mM caused significant increase in growth parameters, namely, plant height, number of branches, number of leaves, shoot and root length total dry biomass [1] and leaf area index [44] of tomato under low temperature stress. Likewise, [45] found that the application of ASA foliar sprays significantly improved various growth parameters (shoot biomass and shoot dry weight) of *P. vulgaris* seedlings (thirty day-old seedlings) exposed to chilling stress for 2 or 4 days.

In the current study, although using ASA and PS, each alone, caused a significant improvement in all vegetative growth of tomato at harvest time, the mix foliar application of both compounds was more efficient than each alone. In this respect the mixed application of both compounds caused a further significant improvement in some vegetative growth characters, namely, leaf area, number of leaves and plant fresh weight.

our results confirmed those of other researchers who proved that foliar application of melatonin (Me) enhanced tomato plant growth under heat and cold stress conditions [46].

Enhancing the vegetative growth characteristics, the foliar applications of the ameliorative substances related to improving chlorophyll b, total carotenoids (Figure 4), non-enzymatic (Table 3) and enzymatic antioxidants (Figure 3) under low temperature conditions as compared with non-treated controls.

Table 2. Effect of some phenolic components, different potassium source and melatonin on vegetative characterization of tomato plants.

Treatments	Height (cm)	Number of branches	Stem diameter (cm)	Leaf area (cm)	Number of leaves	Fresh weight (g)
Season 2019-2020						
Control	47.86 c	7.35 e	2.19 a	79.84 e	228.42 f	1.91 c
ASA	57.15 ab	8.79 bc	1.99 bc	86.09 d	252.25 d	2.16 b
PS	57.98 ab	9.10 abc	1.93 c	94.92 c	282.83 b	2.17 b
Mix	58.97 ab	9.50 a	1.53 e	109.59 a	303.08 a	2.67 a
Melatonin	58.75 ab	8.69 cd	1.97b c	105.97 ab	259.67 c	2.16 b
SA	59.45 a	9.36 ab	1.75 d	102.38 b	286.67 b	2.57 a
PP	56.62 b	8.17 d	2.06 b	84.21 de	235.75 e	2.11 b
Season 2020-2021						
Control	53.30 c	7.58 b	2.24 a	90.03 e	186.00 e	1.41 d
ASA	58.10 abc	8.67 a	1.93 c	104.92 c	316.67 bc	2.57 ab
PS	61.05 ab	9.27 a	1.95 c	112.75 b	323.83 b	2.57 ab
Mix	63.73 a	9.31 a	1.74 d	124.76 a	364.25 a	2.66 a
Melatonin	57.80 bc	9.15 a	2.08 b	104.84 c	269.00 d	2.36 b
SA	61.65 ab	8.73 a	1.78 d	117.70 b	312.17 c	2.39 ab
PP	58.73 abc	8.71 a	2.09 b	98.16 d	175.17 f	1.73 c

ASA: Acetyl salicylic acid, PS: Potassium silicate, Mix: ASA + PS, SA: Salicylic acid and PP: Potassium phosphonate.

c. Antioxidant Enzyme Activity.

In response to cold stress, reactive oxygen species (ROS) like singlet oxygen ($1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals are produced, which cause damage to lipids, proteins, carbohydrates, and nucleic acids [47]. The freezing point within the cell decreases due to the accumulation of osmolytes (such as sugars, polyalcohols, amino acids, polyamines, quaternary ammonium compounds, etc.) that are induced by low temperatures [48]. In order to resist the effects of cold stress and preserve growth and development, plants have developed a number of techniques [49].

The present results revealed that spray treatments with ASA, SA and Mix significantly enhance activity of Catalase (CAT), Peroxidase (POD) and Polyphenol oxidase (PPO) enzymes as compared with non-treated control (Figure 3a, b, c).

Plants have developed a complex antioxidant system to maintain homeostasis using enzymatic antioxidants [superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX)] as one of the methods to mitigate and repair the damage caused by cold stress [47]. Catalase (CAT), Peroxidase (POD) and Polyphenol oxidase (PPO) enzymes defend cellular function in plants and alter the expression of scavenger enzymes in the cell membrane system against oxidative damage of salinity stress [50]. We used here in the present study PS, SA, ASA and the mixture of ASA and PS. SA, ASA and the mixture of ASA and PS caused significant increase in CAT, POD and PPO enzymes. It was reported that SA and ASA have similarities in the chemical, physical, and physiological characteristics [45]. ASA and other phenolic compounds (e.g., SA) was found to enhance the chilling tolerance of plants [39], through significant increase in the activities of the antioxidant enzymes APX, SOD, POD, and CAT [45]. One the other hand application of PS showed no remarkable effect on CAT activity as compared with control (Figure 3a). The mixture of ASA and PS showed a remarkable increase in CAT, as compared to PS, and a relative increase in CAT, as compared to ASA. Meanwhile, the activity of POD and PPO in plants treated with ASA, PS and their mixture was similar (Figure 3b, c). Moreover, one of the most essential components of the cell wall (CW), lignin, which contributes only to the chemical defense of the plant against pathogens, insects, and abiotic stress. Modifications in CW characteristics with an increase in lignification during cold stress are one of the plant responses against cold harm. Complex phenolic polymer lignin improves plant CW stiffness and serves as a crucial barrier to guard cells from the harmful effects of numerous biotic and abiotic factors [51]. The remarkable improvement in plant growth obtained from using the mixture of ASA and PS may be due to increasing the formation of lignin, which is synthesized urgently by the phenolic compound, like ASA [52] and increasing lignin deposition due to presence of PS, as was noticed in rice [53] and tobacco leaves [54].

The effect of Me on enzyme activity was not constant. Me induced CAT in both seasons, PPO in the second season, but reduced POD in the second season. The investigation of [55], reported that the exogenous application of Me substantially increased the activities of CAT and POD, in tomato under cold stress conditions. Such results indicated that increased activity of CAT may be used as an indicator for enhancement of Me to tomato plants to cold stress. On the other hand, the effect of PP on enzyme activity was not significant. This may reveal the reason of lowest effect of PP on plant improvement under cold stress condition. However, [56] observed that the activities of CAT and SOD in plants treated with phosphate during heat stress, were much higher than in those who were not treated with phosphate.

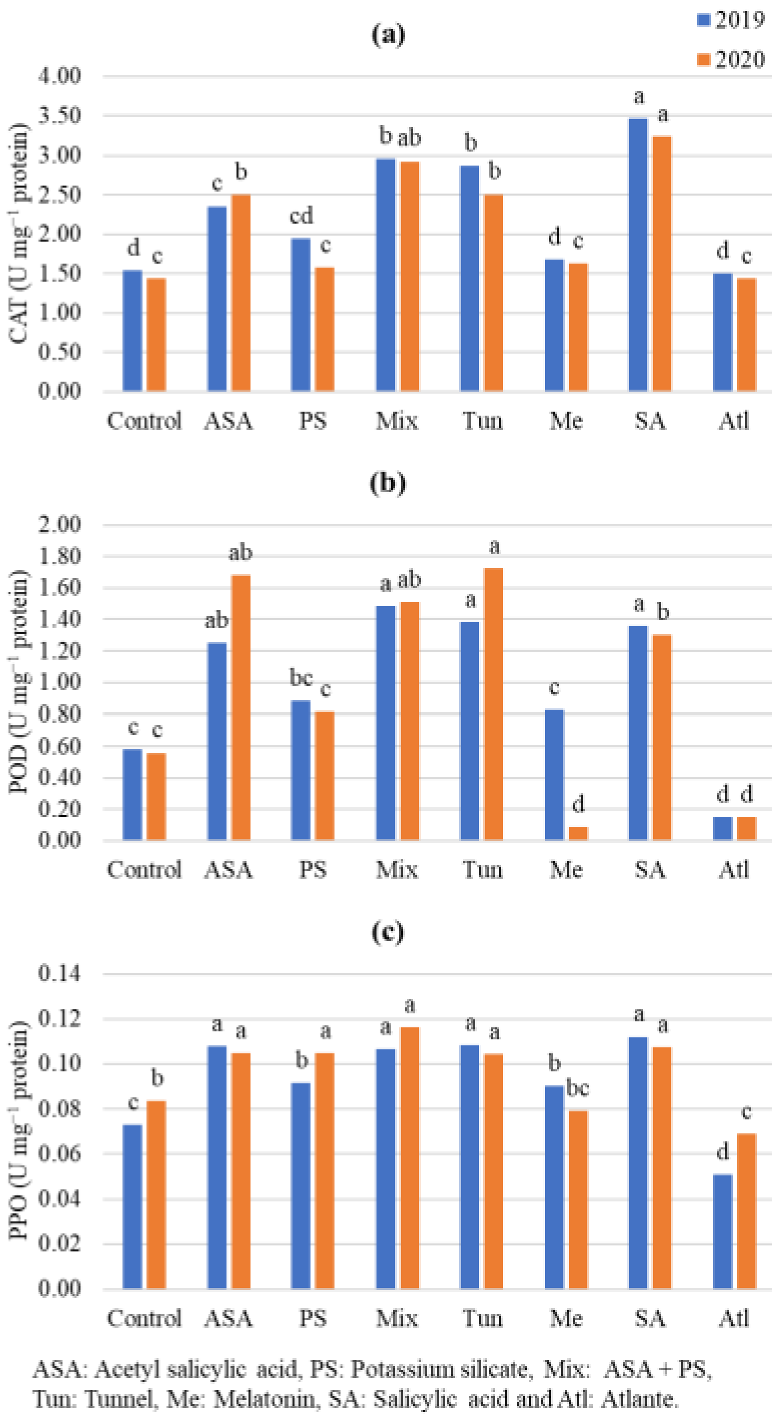

Figure 3. Antioxidant enzyme activities in tomato after treatments. (a) catalase, CAT (U mg^{-1} protein); (b) peroxidases, POD (U mg^{-1} protein) and (c) Polyphenol oxidases, PPO (U mg^{-1} protein).

d. Antioxidant non-Enzymatic compounds (Osmolytes).

Osmoprotectants are substances that are dissolved in a cell's solution or other liquids and are important for regulating the proportion of water in a cell [57]. Various osmoprotectants have been identified, like proline, glycine betaine, TMAO, sarcosine, soluble sugars and sugar alcohol, taurine, , myo-inositol and others [58]. These osmoprotectants defend against different stresses primarily by eliminating reactive oxygen species, preserving protein or enzymatic equilibrium, controlling osmosis, and preserving membrane integrity [59].

The present results revealed that ASA significantly decreased all non- Enzymatic Antioxidants. Similarly, SA and PP significantly decreased all non- Enzymatic Antioxidants, except total indoles, which were increased. Likewise, PS decreased all non- Enzymatic Antioxidants, except Total phenols, which were increased. Meanwhile, total indoles, total phenols and total sugars declined as a result of the foliar application with melatonin, which enhanced the leaf content of total free amino acid. In contrast, there was increase in the leaves content of total free amino acid and total indole due to using mixed treatments, while this treatment caused a reduction in the rest of non- Enzymatic Antioxidants (total phenols, proline and total sugars) (Table 3). These results indicated that tomato plants withstand cold stress by maintenance of high levels of different non- Enzymatic Antioxidants, namely, total indoles by SA and PP, total phenols by PS, total free amino acid by melatonin, total free amino acid and total indole by the mixed treatment between ASA and PS.

The current results are in accordance with the previous studies that revealed that the spraying the plants with PS increased the total phenolic content, as in strawberry leaves [60] and zucchini fruits under greenhouse condition of [61]. Also, SA is a crucial phenolic signaling compound related to plant growth and resistance to biotic and abiotic stresses [21,62].

On the contrary, as compared with the control, total sugars and proline were significantly lower in all treatments, except Melatonin treatment that had a similar level of proline as the control (Table 3). The decrease in the total sugars and proline and the non-significant effect of the different treatments on the activity the some osmoprotectants, as we expected, can be explained by the fact that the effect of these alleviators depended on the time of application, the stress type and plant species [63].

Table 3. Effect of treatments on antioxidant non enzymic compounds in tomato leaves.

Treatments	Total free amino acid (g./100g. DW)	Total indoles (mg/100g. DW)	Total phenols (% DW)	Proline (mg/100g. DW)	Total sugars (% DW)
Season 2019-2020					
Control	0.77 c	10.68 b	0.99 b	24.99 a	19.44a
ASA	0.65 d	9.31 c	0.82 cd	15.44 c	16.22c
PS	0.53 e	9.46 c	1.06 a	12.91 d	11.93d
Mix	0.92 a	11.62 a	0.79 de	12.05 d	7.79e
Melatonin	0.84 b	9.52 c	0.66 f	23.65 a	11.73d
SA	0.62 d	11.88 a	0.85 c	16.50 c	17.88b
PP	0.48 f	12.20 a	0.78 e	19.32 b	8.44e
Season 2020-2021					
Control	0.76 c	11.02 b	0.99 b	26.66 a	19.77a
ASA	0.66 d	8.98 c	0.82 d	15.11 cd	15.55c
PS	0.53 f	9.13 c	1.09 a	12.57 de	11.46d
Mix	0.92 a	11.95 ab	0.78 de	11.38 e	8.12e
Melatonin	0.84 b	9.85 c	0.66 f	24.65 a	12.40d
SA	0.63 e	12.21 a	0.86 c	16.17 bc	17.21b
PP	0.47 g	12.53 a	0.78 e	17.98 b	8.67e

ASA: Acetyl salicylic acid, PS: Potassium silicate, Mix: ASA + PS, SA: Salicylic acid and PP: Potassium phosphonate.

e. Chlorophyll and Carotenoid Content.

Chlorophyll fluorescence measurement has been successfully used as a non-invasive technique to measure plant responses to different abiotic stress [64]. Carotenoids are developed by plants as a complex antioxidant system to maintain homeostasis to alleviate and repair the damage of cold stress [47]. It was clear from the present study that ASA, Mix and Me caused a significant increase in chlorophyll b, and total carotenoids. Mix treatment between PS and ASA showed higher chlorophyll b value than using ASA alone, despite on non-significant effect of using PS alone on this trait (Figure 4b, c).

The antioxidant system's activity is most likely greatly increased by the exogenous administration of ASA, directly affecting the level of photosynthetic activity. [65] This was demonstrated by [66] who found that ASA greatly increased the biomass of *Solanum bulbocastanum* in Vitro under salt stress as a result of an increase in chlorophyll and carotenoids contents and the flow of antioxidant enzyme activity, which reduces oxidative stress and improves photosynthetic activity.

The best treatment was Me similarly, [67] in a recent study, observed that Me treatment effectively increased the carotenoid content and chlorophyll pigment molecules in the leaves of pepper plants under the stress of low temperature and low light. Application of melatonin in the current study may prevent damage caused by stress on chlorophyll, where it increased chlorophyll b content. [68] reported that Me protects the thylakoid membrane from damage induced by low temperature stress by decreasing the damage to the photosynthetic apparatus and increasing the electron transfer rate and quantum yield of photosystem I (PSI) and photosystem II (PSII) photochemistry. Interestingly, in contradiction with our results, many previous studies proved increases in leaf chlorophyll content due to application of PS [28] and SA [65] under salinity stress conditions.

On the other hand, there isn't an obvious difference between the treatments in chlorophyll a (Figure 4a).

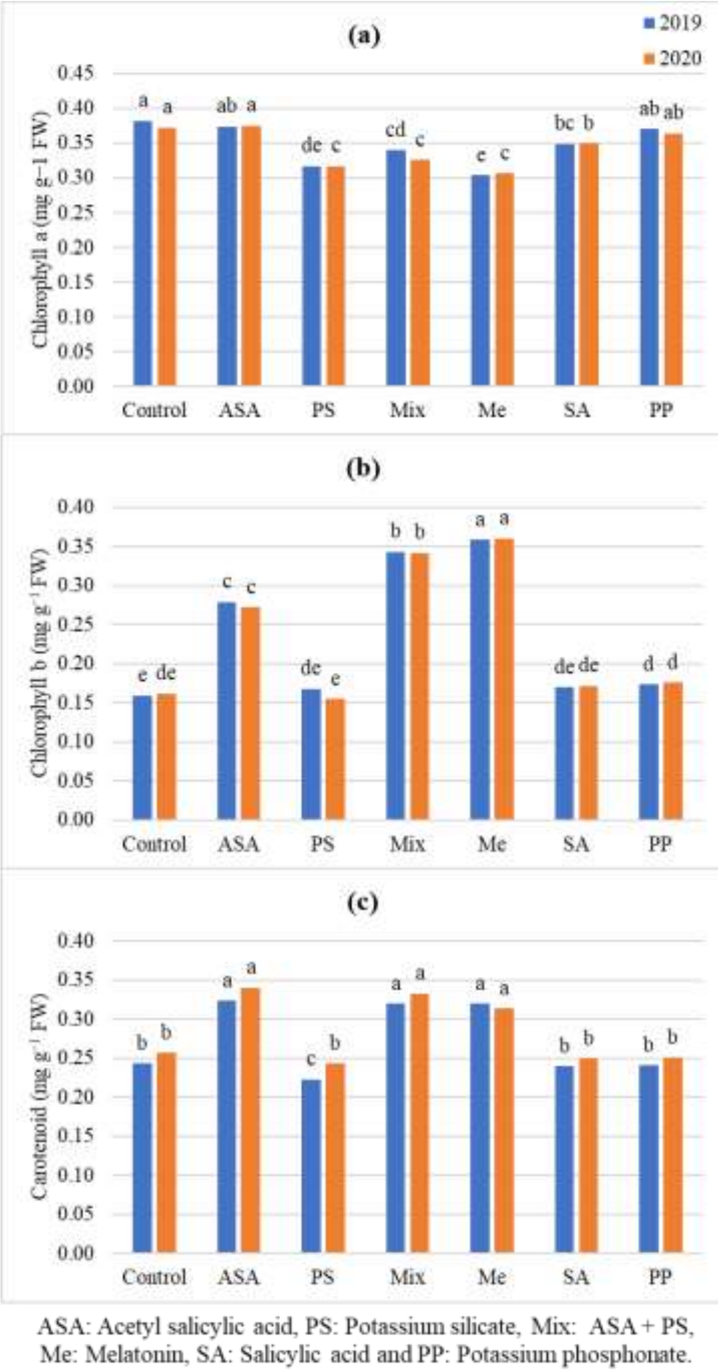

Figure 4. Effect of foliar application treatments on (a) chlorophyll a; (b) chlorophyll b; and (c) carotenoid content in tomato (mg g^{-1} FW).

f. Fruit set percentage and Yield.

Our results showed that cold alleviating compounds significantly increased fruit set, and yield. Mix treatment showed the highest values of early yield, total yield, number of fruits/plant and total yield/plant. Meanwhile, this treatment significantly surpassed ASA and PS each alone. SA and Me came in the second order in this respect (Table 4).

These results might be attributed to the role of these compounds in mitigating cold stress, by stimulation of plant defense through raising the enzymatic (Figure 3), non-enzymatic activity (total free amino acid and total indoles) (Table 3), chlorophyll b; and carotenoid content (Figure 4) that led to improving the vegetative growth of tomato plants. The improving the vegetative growth of tomato plants (source) is related to the tomato fruits (sink).

The high yield presented here is in line with earlier research on the beneficial effects of PS that increased yield in strawberries [69], squash [70] and tomato [26], grown under water stress conditions.

Similarly, ASA treatment improved yield of tomato, [1,44] under chilling stress. Application of ASA could be used as a growth regulator to increase tomato plant resistance to low night-time temperatures stress and increase fruit yields [71]. Previous studies also revealed the importance of using SA in enhancement of low temperature tolerance and improvement of yield in pepper [72], cucumber [15], and peas [73] tomato [1,44].

Table 4. Effect of treatments on fruit set percentage and tomato yield.

Season 2019-2020					
Treatments	Fruit set %	Early yield (Ton/Fed)	Total yield (Ton/Fed)	Number of fruits / plants	Yield (kg/plant)
Control	76.32 d	9.81 c	20.70 d	75.33 d	6.76 e
ASA	84.62 ab	12.72 a	25.15 b	95.92 b	10.53 c
PS	81.33 bc	12.17 ab	26.11 b	93.33 b	9.58 d
Mix	86.67 a	12.91 a	27.80 a	113.58 a	13.64 a
Melatonin	82.73 b	11.15 bc	23.22 c	87.75 c	9.48 d
SA	84.88 ab	12.37 ab	26.24 ab	116.67 a	11.36 b
PP	78.58 cd	11.31 b	21.95 cd	86.58 c	9.31 d
Season 2020-2021					
Control	64.02 e	11.33 d	22.11 f	74.67 c	6.83 e
ASA	93.45 a	12.91 bc	24.95 c	93.33 b	8.43 d
PS	80.96 c	13.54 ab	27.16 b	115.25 a	11.87 a
Mix	75.65 d	13.64 a	28.27 a	116.50 a	12.10 a
Melatonin	82.11 bc	12.51 c	24.12 d	94.25 b	10.81 b

SA	83.96 b	13.71 a	28.26 a	77.00 c	9.94 c
PP	76.65 d	11.73 d	23.25 e	66.83 d	7.32 e

ASA: Acetyl salicylic acid, PS: Potassium silicate, Mix: ASA + PS, SA: Salicylic acid and PP: Potassium phosphonate.

g. Chemical fruit quality

Except for Total Soluble Solids (%), which was not affected by Me and PS in the first season, all treatments significantly improved fruits chemical quality, i.e., Lycopene, Carotene, Vitamin C (Vit. C), and Total soluble solids (TSS) but they reduced fruit acidity.

The maximum lycopene contents were observed in plants treated with Mix and SA. The most effective treatments on β-carotene content were Mix and PS (Table 5). Lycopene, carotene and Vit. C (ascorbic acids) are non-enzymatic antioxidants, that are adversely affected by low temperature stress [44]. Our findings are supported by the findings of other studies that showed that foliar application of SA enhance the lycopene content [74,75] and Vit. C [74] content in tomato, and eggplant [76]. Lycopene contents may have increased as a result of SA's activation to the lycopene biosynthesis pathway during fruit development, including up-regulation of the genes encoding the enzymes that regulate lycopene levels. Similarly, another study proved that SA increased β-carotene content in tomato fruits [75]. Likewise, [44] reported that foliar applications of SA, ASA significantly enhanced Vit. C, lycopene and β-carotene content in tomato under low temperatures conditions. The increase in carotene and lycopene in our study due to application of PS is in harmony with the previous ones that recorded increase in lycopene and β-carotene in tomato fruits, when tomatoes were grown in hydroponics [77], under deficit irrigation [26] compared with the untreated plants (water). Similar results were found by [78] They reported the effectiveness of the use of foliar sprays containing stabilized silicic acid and observed an increase in lycopene and ascorbic acid. As our findings, PS treatment increased Vit. C in tomato fruits [77,79] and sweet pepper [80]. [81] found that Me enhances the carotenoids biosynthesis in tomato under stress conditions. Moreover, Me may play a significant function in modulating the development of pigment in fruits. Under the circumstances of this study, all of these effects could explain the significant influence of applied Me on the fruit's qualitative qualities.

The maximum TSS were recorded in Mix followed by SA, the lowest content in Me. Other investigations concurred with our findings, reporting that foliar SA sprays raised TSS in tomato fruits grown in greenhouses [82] and under low temperature conditions [83]. The main factor is likely because SA stimulates the production of hydrogen peroxide, which in turn greatly increases the activity of the enzyme phenylalanine ammoniumlyase, which is in charge of producing phenolic compounds [84]. Foliar applications of ASA significantly enhanced TSS in tomato fruit under cold stress condition [1] and under salt stress [85]. [70] reported that foliar application of Si enhancement TSS in squash fruits under deficit irrigation condition. [79] reported that foliar application with PS increased TSS in three winter, tomato cultivars. When compared to untreated plants, Mix and SA significantly decreased the amount of titratable acidity. In tomatoes, titratable acidity dropped as the temperature decreases [86]. Spraying tomato with SA, ASA [1] or PS [79] under low temperature as well as soil application of Si [87] decreased the amount of titratable acidity in tomato fruits irrespective of the stage of plants.

Table 5. Effect of foliar applications on tomato chemical fruit quality.

Season 2019-2020					
Treatments	Lycopene (g./100g)	β-Carotene (g./100g)	Total Soluble Solids (%)	Acidity (%)	Vit.C (mg/100g)
Control	0.34 f	0.23 d	3.83 d	0.30 a	8.81 d
ASA	0.47 d	0.44 b	4.80 bc	0.25 c	10.89 c
PS	0.51 c	0.55 a	4.57 c	0.22 d	15.59 a
Mix	0.67 a	0.56 a	5.77 a	0.19 f	14.93 a
Melatonin	0.43 e	0.34 c	4.17 cd	0.26 bc	9.97 c
SA	0.54 b	0.46 b	5.33 ab	0.21 e	12.58 b
PP	0.42 e	0.33 c	4.30 cd	0.27 b	10.01 c
Season 2020-2021					
Control	0.37 f	0.23 e	4.03 d	0.3 a	8.55 f
ASA	0.52 d	0.42 c	5.50 b	0.26 bc	11.57 c
PS	0.56 c	0.54 a	5.00 c	0.24 cd	15.50 a
Mix	0.75 a	0.57 a	6.40 a	0.18 e	15.42 a
Melatonin	0.44 e	0.33 d	5.00 c	0.28 b	10.78 d
SA	0.60 b	0.46 b	5.87 b	0.22 d	13.49 b
PP	0.47 e	0.31 d	4.67 c	0.27 b	9.90 e

ASA: Acetyl salicylic acid, PS: Potassium silicate, Mix: ASA + PS, SA: Salicylic acid and PP: Potassium phosphonate.

4. Conclusion

It is suggested that ASA and PS had synergetic effect. They jointly induced increase in photosynthetic capacity of tomato plants under low temperature stress.

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