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Phenolic Compounds Enhanced Low Temperature Stress Tolerance in Tomato (*Solanum lycopersicum* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author YKM performed the experiment, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DSK, NK and KS designed and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Low temperature stress adversely affects plant growth and development and it directly affects the yield and quality of tomato. Phenolic compounds have been implicated to mitigate cold stress. Therefore an experiment was conducted to find out suitable concentration of phenolic compounds to ameliorate effect of low temperature stress in tomato under open field conditions during winter season of 2014-15 and 2015-16. The average minimum temperatures were below 10°C from December 15 to February 15 for both the years of study. The experimental plants were given foliar application twice, first 15 days after transplanting followed by another spray after a fortnight of phenolic compounds viz., salicylic acid, sulfo salicylic acid, benzoic acid, methyl salicylic acid and acetyl salicylic acid at different concentration, *i.e.*, 0.1 mM, 0.5 mM & 1.0 mM in order to evaluate

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their effect on morphological, yield and quality traits. Based on two year study, its observed that low temperature significantly reduces the growth traits (plant height, number of branches, number of leaves, shoot and root length, and total biomass of plant), yield attributes (days to 50% flowering, fruit weight and fruit yield) and quality parameter (total soluble solid), however there was an increase in titrable acidity and ascorbic acid. On the hand, application of phenolic compounds significantly enhanced the growth, yield and TSS, while, decreased titrable acidity and ascorbic acid under stress. Two years study has confirmed that phenolic compounds protect plants against low temperature stress and enhanced production of tomato with an increase in the yield and quality contributions attributes. Among the treatments, SA (1.0 mM) was found as most effective to enhance low temperature stress tolerance in tomato.

Keywords: Growth; yield; quality; low temperature stress tolerance; tomato.

1. INTRODUCTION

Vegetables have an important place in the diversification of agriculture and have played a vital role in food and nutritional security of ever growing population of India. However, vegetables are sensitive crops and their production is limited by various abiotic stress factors. Temperature, both low and high, is by far the most serious environmental stress limiting crop production. Low temperature stress is one of the most restraining environmental factors for agricultural crops especially vegetables, which accounts for significant crop losses [1]. Tomato is one of the most common and widely grown vegetable in the world, ranks second in importance only after potato [2]. Tomato plants are sensitive to chilling temperature (0-15°C) throughout their development *i.e.* during seed germination, vegetative growth and reproduction. Under low temperature, lot of seeds do not germinate or germinate irregularly so that the plants grow differentially with delayed plant formation which leads to variability in crop maturation. Chilling stress during later stages of plant growth and development leads to extremely retarded growth that either limitor lead to no flower and fruit production [3].

Plant hormones play essential roles in regulation of developmental processes and signaling networks in plants suffering from abiotic stresses [4,5]. Phenolic compounds have been recently added to the known classical plant hormones and have shown as possible tools in boosting tolerance of plants to abiotic stress. They are believed to have role in plant responses to abiotic stresses including low temperature stress [5,6]. Salicylic acid (SA) and other phenolic compounds such as benzoic acid, acetyl salicylic acid, sulfo salicylic acid have been found to increase the cold tolerance of plants when applied exogenously [7]. Application of phenolic compounds assists in improving the tolerance against low temperature stress in plants. Moreover, less is known about the suitable concentration of phenolic compounds to mitigate low temperature stress in tomato. Besides this, field appraisals of exogenously applied phenolic compounds are also lacking. This study was meant to figure out the effect of foliar application of phenolic compounds on morphology, yield and quality related traits of tomato plants at low temperature stress.

2. MATERIALS AND METHODS

2.1 Hormone Preparation

Salicylic acid (SA), sulfo salicylic acid and benzoic acid were obtained from Sisco Research Laboratories Pvt Ltd. 26, Novketan Ind. M C Rd. Andheri (E), Mumbai-400099, India. Acetyl salicylic acid (ASA) and methyl salicylic acid were obtained from Cyman Chemical Company, 1180 East Ellsworth Road Ann Arbor, Michigan 48108 (USA) and Himedia Laboratories Pvt Ltd. LBS Marg Mumbai 400086 (India), respectively. Stock solution (2 mM) was prepared by dissolving the required quantity in 2 ml of ethanol, in a 1000 ml volumetric flask, and the final volume was made up to mark by using double distilled water (DDW). The concentrations (0.1, 0.5 and 1.0 mM) of above mentioned phenolic compounds were prepared by dilution of stock solution in distilled water.

2.2 Plant Material and Stress Conditions

Seeds of tomato variety Punjab Ratta were obtained from Department of Vegetable Science, Punjab Agricultural University, Ludhiana, India. Seeds were sown in nursery plug tray and covered with polyethylene to protect from low temperature. 28 days after sowing, seedlings were transplanted on 1st December of 2014 and 2015 under open field conditions; the average minimum and maximum temperatures of both years are shown in Fig. 1. The cultural practices used were as per Punjab Agricultural University recommended practices. Foliar application of phenolic compounds (0.1, 0.5 and 1.0 mM) were given 15 and 30 days after transplanting; in one treatments plants protected by shelterbelt (Covered by polyethylene, as per recommended by Punjab Agricultural University) [8] were kept and in control plants given foliar spray of distilled water. The experiment was designed in complete randomized block design with three replications per treatment. In each plot, 40 plants were sown and 5 plants were randomly selected for growth attributes at 30, 60 and 90 days after transplanting and the remaining were for yield and quality parameters. The titrable acidity and ascorbic acid were estimated by the method of AOAC [9]. Total soluble solid measured by used hand refractrometer at room temperature and readings were expressed as Brix (°B).

2.3 Statistical Analysis

Data was analyzed using STAR 2.0.1 software developed by International Rice Research Institute. The mean comparisons were performed by Duncan's multiple range test at (*P*=.05).

3. RESULTS AND DISCUSSION

3.1 Morphological Investigations

Low temperatures stress adversely affect plant growth and development. Low temperature stress significantly reduced most of the growth biomarkers *viz.,* plant height, number of branches, number of leaves, shoot and root length and total dry biomass of plant at 30, 60 and 90 days after transplanting (DAT) during both the years study as compared to plants protected by shelterbelt (data shown in Tables 1 and 2). The minimum growth biomarkers were observed in non treated plants (control) during both the years of study. Low temperature stress bounds crop yield by reducing plant growth, with negative and irregular effects on biomass accumulation. Similarly, Khan et al. [10] and Sayyari et al. [11] observed that low temperature stress has considerable reduction in fresh and dry mass of root, shoot and root length and shoot in tomato and watermelon, respectively. Low temperature caused tissue discoloration and increased water loss as a result of suppressed expression of genes which are generally active at normal temperatures [12]. In addition to this, [13] reported quick decline in the number of dividing cells during low temperature; it decreased the mitotic index in apices and in the basal part of young leaves. Cell growth was inhibited which caused considerable changes in plant organs [14].

On the other hand, foliar applications of phenolic compounds significantly enhanced plant growth attributes viz., plant height, number of branches, number of leaves, shoot and root length and total dry biomass of plant at different growth stages during both year studies. Among the phenolic compounds, SA (1.0 mM) was most effective followed by ASA (0.5 mM), SSA (1.0 mM) and ASA (1.0 mM). However SA (1.0 mM) was statistically at par with plant protected by shelterbelt. Foliar application of SA increased the level of cell division by stimulating the mitotic system of the apical meristem of seedling roots which caused an increase in plant growth [15]. A support to the present study, Orabi et al. [16] reported significant increase in growth parameters like plant height, number of leaves per plant, fresh and dry weights of leaves and root length with the application of SA under low temperature stress in tomato. Similarly results obtained by [11] in watermelon and [17] in chickpea showed that the growth parameters significantly increased with the application of SA under cold stress condition. The ability of SA to enhance growth parameters, ameliorating the adverse effects of cold stress, may have significant implications in increasing the plant growth and development, and overcoming the growth barrier. According to Gharib [18] enhanced photosynthetic activity in basil with the application of SA at low concentration, enhanced their plant growth attributes *viz.,* plant height, number of branches and leaves per plant as well as leaf area, fresh and dry weights of plants. Similarly results reported by [19] showed that application of SA significantly enhanced growth parameters such as; length and dry weight of root and shoot under stress conditions [20,21]. Hussein et al. [22] and Hayat et al. [23] also reported increased productivity due to an improvement in growth attributes including plant height, number of leaves, leaf area, dry and fresh weight of shoot, leaves and plant with the application of SA and sulfo salicylic acid under stress.

Table 1. Effect of phenolic compounds on plant height, number of branches and number of leaves in tomato under low temperature stress

Treatments	Plant height (cm)			Number of branches			Number of leaves		
	30DAT	**60DAT**	**90DAT**	**30DAT**	**60DAT**	**90DAT**	**30DAT**	**60DAT**	**90DAT**
SA (0.1 mM)	12.79^{cde}	24.07^{fg}	40.08^{ef}	4.93^{ef}	8.58^{ef}	14.90^{d}	17.26^{defg}	107.22^{hi}	249.55^{ghi}
SA (0.5 mM)	13.59^{bcde}	26.74^{b-f}	43.59^{b-f}	5.29^{a-f}	8.96^{cdef}	16.05^{abcd}	19.32^{bcd}	126.09^{def}	274.92^{efg}
SA (1.0 mM)	15.22^{a}	29.60^{ab}	48.51^{ab}	5.68^{ab}	9.84^{ab}	17.15^{a}	21.59^{a}	138.58^{ab}	339.30^{ab}
MSA (0.1 mM)	12.29^{e}	23.52^{g}	38.55^{f}	4.80^{f}	8.37^{ef}	14.71^{d}	16.47^{g}	103.53^{i}	242.78^{hi}
MSA (0.5 mM)	13.28^{bcde}	25.59^{defg}	42.67^{cdef}	5.06^{c-f}	8.62^{ef}	15.37^{bcd}	18.95^{cdef}	119.24^{fg}	262.16^{fghi}
MSA (1.0 mM)	13.86^{abcd}	27.45^{a-e}	44.16^{a-e}	5.35^{a-f}	8.98^{b-f}	16.16^{abcd}	20.24^{abc}	127.23^{cdef}	295.07^{de}
SSA (0.1 mM)	12.74^{cde}	23.97^{fg}	40.02^{ef}	4.98^{def}	8.53^{ef}	14.96^{cd}	17.12^{efg}	109.67^{hi}	248.15^{hi}
SSA (0.5 mM)	13.56^{bcde}	26.94^{a-e}	43.84^{a-f}	5.21^{b-f}	8.81^{def}	16.00^{abcd}	19.30^{bcd}	126.10^{def}	278.60^{ef}
SSA (1.0 mM)	14.75^{ab}	28.75^{abc}	47.70^{abc}	5.53^{abcd}	9.60^{abc}	16.79^{ab}	21.90^{abc}	135.32^{abcd}	327.96^{abc}
BZA (0.1 mM)	12.44^{de}	23.46^{g}	39.48^{ef}	4.85^{f}	8.29^{f}	14.80^{d}	16.99^{fg}	105.69^{i}	241.73^{i}
BZA (0.5 mM)	13.41^{bcde}	25.96^{c-g}	42.95^{cdef}	5.11^{b-f}	8.72^{def}	15.46^{bcd}	19.13^{cde}	122.28^{efg}	268.86^{fgh}
BZA (1.0 mM)	14.15^{abc}	27.65^{a-e}	44.88^{a-e}	5.39^{a-f}	9.14^{bcde}	16.40^{abcd}	20.60^{abc}	129.12^{bcde}	306.69^{cd}
ASA (0.1 mM)	12.88^{cde}	25.09^{efg}	40.92^{def}	4.99^{def}	8.74^{def}	15.00^{cd}	17.65^{defg}	116.12^{gh}	253.71^{fghi}
ASA (0.5 mM)	15.06^{a}	29.15^{ab}	48.02^{abc}	5.61^{abc}	9.74^{ab}	16.91^{ab}	21.36^{ab}	136.51^{abc}	333.49^{ab}
ASA (1.0 mM)	14.65^{ab}	28.04^{abcd}	46.10^{abcd}	5.48^{a-e}	9.41^{abcd}	16.61^{abc}	20.83^{abc}	133.55^{abcd}	318.39^{bcd}
Shelterbelt	15.27^{a}	29.75^{a}	48.99^{a}	5.87^{a}	9.95^{a}	17.34^{a}	21.77^{a}	140.19^{a}	346.03^{a}
Control (DW)	9.60^{f}	18.56^{h}	32.91^{g}	3.91^{g}	6.65^{g}	11.05^{e}	11.49^{h}	81.70^{j}	170.79^{j}

Significant at 5 percent level (Same letter in a given column did not differ significantly as per Duncan multiple range test)

Table 2. Effect of phenolic compounds on shoot and root length and total dry biomass in tomato under cold stress conditions

Treatments	Shoot length (cm)			Root length (cm)			Total dry biomass plant^{-1} (g)		
	30DAT	**60DAT**	**90DAT**	**30DAT**	**30DAT**	**90DAT**	**30DAT**	**60DAT**	**90DAT**
SA (0.1 mM)	10.73^{efg}	20.01^{fgh}	36.01^{hi}	7.47^{efg}	12.20^{ef}	17.22^{fgh}	1.15^{ab}	7.68^{gh}	67.06^{f}
SA (0.5 mM)	11.36^{b-f}	22.40^{cde}	39.90^{d-h}	8.26^{a-e}	13.31^{cde}	19.40^{e}	1.37^{abc}	9.23^{ef}	77.17^{e}
SA (1.0 mM)	12.42^{ab}	25.26^{ab}	45.94^{ab}	8.94^{ab}	15.45^{a}	24.87^{ab}	1.64^{ab}	10.78^{ab}	114.74^{ab}
MSA (0.1 mM)	10.45^{g}	19.03^{h}	35.04^{i}	7.18^{g}	11.83^{f}	16.69^{h}	1.02^{b}	7.14^{h}	63.20^{f}
MSA (0.5 mM)	11.04^{c-g}	21.51^{defg}	38.43^{fghi}	8.01^{c-g}	12.81^{def}	18.51^{efgh}	1.28^{ab}	8.79^{f}	73.90^{e}
MSA (1.0 mM)	11.62^{a-g}	23.02^{bcd}	41.85^{b-f}	8.44^{abc}	13.75^{cd}	21.27^{d}	1.43^{abc}	9.73^{de}	92.64^{d}
SSA (0.1 mM)	10.78^{efg}	19.73^{fgh}	36.20^{hi}	7.42^{fg}	12.14^{ef}	17.15^{fgh}	1.14^{bc}	7.73^{gh}	66.62^{f}
SSA (0.5 mM)	11.33^{b-g}	22.56^{cde}	40.49^{c-g}	8.31^{abcd}	13.72^{cd}	19.08^{ef}	1.36^{abc}	9.26^{ef}	79.34^{e}
SSA (1.0 mM)	12.03^{abcd}	24.37^{abc}	44.30^{abc}	8.73^{abc}	14.99^{ab}	23.26^{bc}	1.57^{ab}	10.40^{abc}	109.76^{bc}
BZA (0.1 mM)	10.62^{fg}	19.35^{gh}	35.59^{i}	7.34^{fg}	11.96^{f}	17.05^{gh}	1.11^{bc}	7.42^{gh}	64.64^{f}
BZA (0.5 mM)	11.18^{c-f}	21.83^{def}	39.05^{e-i}	8.14^{b-f}	13.00^{def}	18.75^{efg}	1.34^{abc}	9.02^{f}	75.74^{e}
BZA (1.0 mM)	11.73^{a-f}	23.45^{abcd}	42.65^{a-e}	8.52^{abc}	14.02^{bcd}	21.74^{cd}	1.33^{abc}	9.98^{cd}	96.28^{d}
ASA (0.1 mM)	10.84^{defg}	20.56^{efgh}	36.66^{ghi}	7.54^{defg}	12.34^{fgh}	17.39^{fgh}	1.17^{abc}	8.01^{g}	68.20^{f}
ASA (0.5 mM)	12.20^{abc}	24.75^{abc}	45.45^{ab}	8.82^{abc}	15.24^{ef}	24.19^{ab}	1.6^{ab}	10.59^{abc}	112.63^{b}
ASA (1.0 mM)	11.86^{abcd}	23.86^{abcd}	43.78^{abcd}	8.59^{abc}	14.57^{abc}	22.02^{cd}	1.52^{a}	10.23^{bcd}	105.89^{c}
Shelterbelt	12.56^{a}	25.54^{a}	46.63^{a}	9.05^{a}	15.66^{a}	25.67^{a}	1.71^{a}	10.98^{a}	118.46^{a}
Control (DW)	7.70^{h}	14.72^{i}	29.88^{j}	5.93^{h}	8.80^{g}	13.91^{i}	0.78^{c}	5.78^{i}	44.02^{g}

Significant at 5 percent level (Same letter in a given column did not differ significantly as per Duncan Multiple Range test)

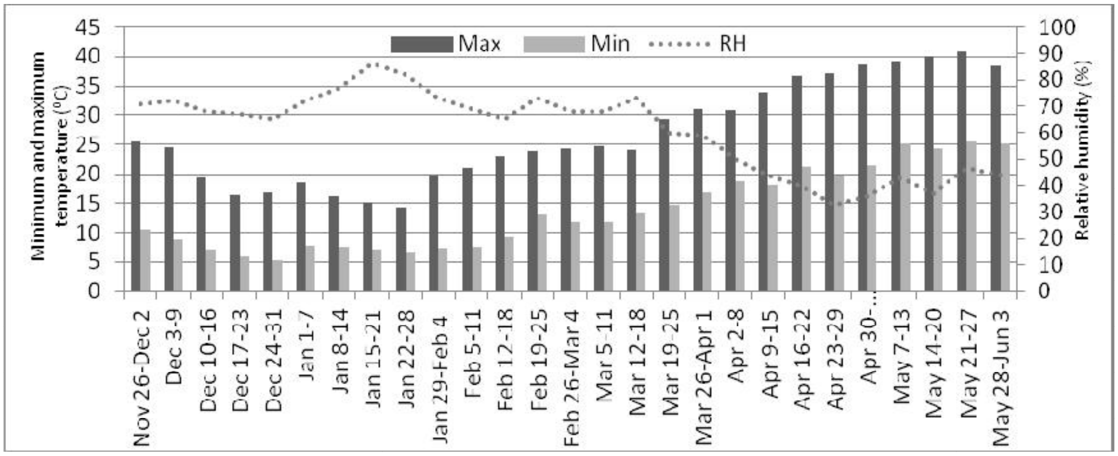

Fig. 1. Average means of minimum, maximum temperature (°C) and relative humidity (%) during 2014-15 and 2015-16

3.2 Yield Contributing Characters

Plants growth under low temperature were affected in terms of days to 50% flowering, with lower average fruit weight and fruit yield as compared to plants protected by shelterbelt. Low temperature stress during vegetative growth significantly reduced the average fruit weight and fruit yield (Table 3). The minimum days to 50% flowering, minimum average fruit weight and minimum fruit yield were observed in non treated plants (control), while the maximum has been observed in non stressed plants (protected by shelterbelt) during both the year of study. Plants grown in the low temperature possessed lower fruit yield in tomato [10] which may be because of the slower CO_2 fixation and partitioning of photosynthates of fruit. However, significant increase in number of days to 50% flowering, average fruit weight and fruits yield per plant recorded with the foliar applications of phenolic compounds under stress. Among all the phenolic compounds, SA (1.0 mM) was more effective as compared to others, it recorded maximum average fruit weight and yield followed by ASA (0.5 mM), SSA (1.0 mM) and ASA (1.0 mM). However SA (1.0 mM) was statistically at par with plant protected by shelterbelt. Two years study has confirmed that phenolic compounds protect plants against low temperature stress and enhanced production of tomato with an increase in the yield contributions attributes. Similarly, research workers reported that the fruit weight and fruit yield increased significantly with foliar application of SA in pepper [23], and cucumber [24].

3.3 Quality Attributes

Total soluble solid (TSS) were affected by low temperature stress during vegetative growth as compared to the plants protected by using shelterbelt as per data shown in Table 3. Low temperature stress significantly enhanced reduction in TSS in stressed plant (4.92°B) as compared to protected plant by shelterbelt (5.85°B). However, foliar applications of phenolic compounds significantly enhanced TSS in tomato fruit under cold stress condition. Among the phenolic compounds, the maximum TSS (5.84°B) was observed in SA (1.0 mM) which was statistically at par with non stressed plants (shelterbelt), ASA (0.05 mM) and SSA (1.0 mM). Application of SA significantly enhanced the TSS in tomato [25]. Mady [26] reported that TSS in tomato fruits increased with the application of SA. Tomato plants treated with salicylic acid had significantly enhanced TSS in tomato [27], cowpea [28]. The application of SA (0.5 mM and 1.0 mM) caused significant increase in TSS in fruits of tomato relative to control plants. TSS values are associated with taste and have significant indication for improvement in yield quality as reported by [16].

The data on titrable acidity and ascorbic acid are shown in Table 3. Titrable acidity and ascorbic acid increased in non treated plants (control) (0.76 g of citric acid ml^{-100} and 24.63 mg ml^{-100} of fresh juice respectively) as compared to plants protected by shelterbelt (0.64 g of citric acid ml^{-100} and 21.27 mg per 100 ml of fresh juice respectively). Increase in titrable acidity and

Table 3. Effect of phenolic compounds on yield and quality in tomato under low temperature stress conditions

Treatments	Days to 50% flowering	Fruit weight (g)	Fruits yield per plant (kg)	Total soluble solid (°B)	Titrable acidity content (g of citric acid/ 100 ml)	Ascorbic acid (mg 100 ml^{-1} of fresh juice)
SA (0.1 mM)	68.74^{h}	55.62^{cde}	1.77^{g}	5.76^{fg}	0.69^{b}	23.18^{cd}
SA (0.5 mM)	71.81^{de}	57.34^{a-e}	2.01^{de}	5.80^{cd}	0.66^{fg}	22.54^{g}
SA (1.0 mM)	73.40^{ab}	59.67^{ab}	2.27^{ab}	5.84^{a}	0.65^{h}	21.30^{hi}
MSA (0.1 mM)	67.97^{ij}	54.73^{e}	1.71^{g}	5.75^{g}	0.68^{bc}	23.40^{b}
MSA (0.5 mM)	70.07^{g}	56.78^{a-e}	1.96^{ef}	5.78^{de}	0.67^{de}	22.87^{ef}
MSA (1.0 mM)	73.06^{bc}	58.25^{a-e}	2.09^{cde}	5.80^{cd}	0.66^{g}	21.47^{h}
SSA (0.1 mM)	69.88^{g}	55.81^{bcde}	1.77^{g}	5.75^{fg}	0.68^{cd}	23.23^{bc}
SSA (0.5 mM)	71.02^{f}	57.41^{a-e}	2.01^{de}	5.80^{cde}	0.67^{ef}	22.70^{fg}
SSA (1.0 mM)	73.23^{b}	59.17^{abcd}	2.21^{abc}	5.83^{ab}	0.66^{h}	21.32^{hi}
BZA (0.1 mM)	67.48^{j}	55.38^{de}	1.74^{g}	5.76^{fg}	0.69^{b}	23.27^{bc}
BZA (0.5 mM)	69.93^{g}	57.13^{a-e}	1.96^{ef}	5.79^{de}	0.68^{cd}	22.80^{f}
BZA (1.0 mM)	71.58^{ef}	58.41^{a-e}	2.11^{bcde}	5.81^{cd}	0.66^{fg}	21.44^{hi}
ASA (0.1 mM)	68.29^{hi}	55.57^{de}	1.81^{fg}	5.77^{ef}	0.68^{cd}	23.00^{de}
ASA (0.5 mM)	74.07^{a}	59.46^{abc}	2.25^{abc}	5.85^{a}	0.65^{h}	21.34^{hi}
ASA (1.0 mM)	72.47^{cd}	58.83^{abcd}	2.16^{abcd}	5.82^{bc}	0.67^{g}	21.40^{hi}
Shelterbelt	73.28^{b}	60.08^{a}	2.30^{a}	5.85^{a}	0.64^{i}	21.27^{i}
Control (DW)	63.67^{k}	38.76^{f}	0.88^{h}	4.92^{h}	0.76^{a}	24.63^{a}

Significant at 5 percent level (Same letter in a given column did not differ significantly as per Duncan multiple range test)

ascorbic acid in tomato fruit may be due to increasing temperature during fruit development. On the other hand, foliar applications of phenolic compounds have significantly reduced the amount of titrable acidity and ascorbic acid as compared to non treated plants. Among all phenolic compounds, SA (1.0 mM) and ASA (0.5 mM) were more effective, being statistically at par with plants protected by shelterbelt. Titratable acidity decreased with decreasing temperature in tomato [29]. But some worker have reported that heat stress during fruit development enhanced the titrable acidity and ascorbic acid in tomato. The increased value may be due to high temperature stress during fruit development. However, the values were decrease with the application of SA.

4. CONCLUSION

To conclused, low temperature stress results in significant reduction in the plant growth, development, yield and quality of tomato. However, application of phenolic compounds reduced the adverse affect of low temperature and enhanced the growth, development, yield and quality of tomato. SA (1.0 mM) was most effective phenolic compound and significantly increased the plant growth, development, yield and quality of tomato cultivar Punjab Ratta. Hence SA (1.0 mM) may be given as foliar application twice at 15 and 30 days after transplanting to tomato in order to mitigate the effect of cold stress. Salicylic acid may be the signalling molecule that inhitiates the stress tolerance cascade resulting in metabolic regulation leading to alleviation of stress tolerance. This being a cost effective measure may prove economical to the tomato growing farmers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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