Journal of King Saud University – Science 35 (2023) 102660

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Foliar spray of salicylic acid and ascorbic acid ameliorates the biochemical compounds in hybrid chillies

Anam Zahid [a], Gao yike [a,*], Anam Abdul Razzaq [b], Muneeb Munawar [c], Fozia [a], Muhammad Ramzan [d], Bader O. Almutairi [e], Mikhlid H. Almutairi [e]

[a] School of Landscape Architecture and Ornamental Horticulture, Beijing Forestry University, PR China
[b] Rural Health Center (RHC) 6/G Tehsil Chishtian, Bahawalnagar, Pakistan
[c] Vegetable Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan
[d] School of Forest Sciences, University of Eastern Finland, Yliopistokatu 7. P.O. Box 111, 80100 Joensuu, Finland
[e] Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:
Received 11 January 2023
Revised 26 February 2023
Accepted 23 March 2023
Available online 30 March 2023

Keywords:
Biochemical compounds
Enzymes
Ascorbic Acid
Salicylic Acid
Chillies

ABSTRACT

Chillies (*Capsicum annuum)* are a spice crop with great medicinal value of its biochemical ingredients. Its high antioxidant value along with good nutritional properties. Foliar application of Salicylic Acid may stimulate various plant physiological parameter i.e., stomatal activity, ions uptake, seed germination, leave membrane response to electrolytes and growth rate. Salicylic Acid (SA) is known as a molecule that is participated in physiological processes in chillies, plant tolerance and resistance to abiotic and biotic stresses. Ascorbic acid possesses antioxidant properties, has positive impact on flowering and expounds effective plant defense system against pesticide toxicity. Moreover, ascorbic acid is effective chemical to mitigate the destructive impacts of salt stress. All the treatments improved the yield, fruit length and fruit width as compared to control treatment. Although, various treatments, salicylic acid and ascorbic acid combination showed the maximum plant height (80.3 cm), shoot weight (161 g), root weight (47.1 g) and pericarp thickness (1.99 mm) in treatment T9 variety of V4. Results declared that foliar spray of plants growth regulators increased the carbohydrates, protein, fiber and ash content. Results declared that foliar spray of plants growth regulators increased the carbohydrates, protein, fiber and ash contents. Furthermore, Biochemical attributes and Enzymes like proline (19.1%), SOD (1.40ug^{-1}Fw), POD (17.9 ug^{-1}Fw g) and CAT (6.15ug^{-1}Fw) were significantly improved in plants sprayed with salicylic acid (SA) and ascorbic acid (AA) at T9 treatment. The objective of this study was to evaluate the effect of foliar application of ascorbic acid and salicylic acid on the yield and biochemical traits of hybrid chillies. Generally, the highest values were obtained from T9 treatment application of SA and AA combination. Based on these findings, the SA and AA treatments may help alleviate the positive effect on the growth of Chillies.

1. Introduction

Chillies (Capsicum annuum L.) are a member of the Solanaceae family and cultivated all over the globe due to its nutraceutical and economic value (Rahman et al., 2013). It is enriched in vitamins (A, D3, E, C, K, B2 and B12), lipids, minerals (Ca, P, Fe, K), proteins, and volatile compounds i.e., capscinoids. The amount of vitamin C is found in higher magnitude in fresh green chillies compared to citrus fruits and more vitamin A than in comparison with carrots (Chigoziri and Ekefan, 2013). There is multiple consumption of Chilli pepper as salad in green fresh form, in various culinary recipes and value adding products like sauces, stews, soups and usually and a flavoring agent (Amruthraj et al., 2014).(See Fig. 1)

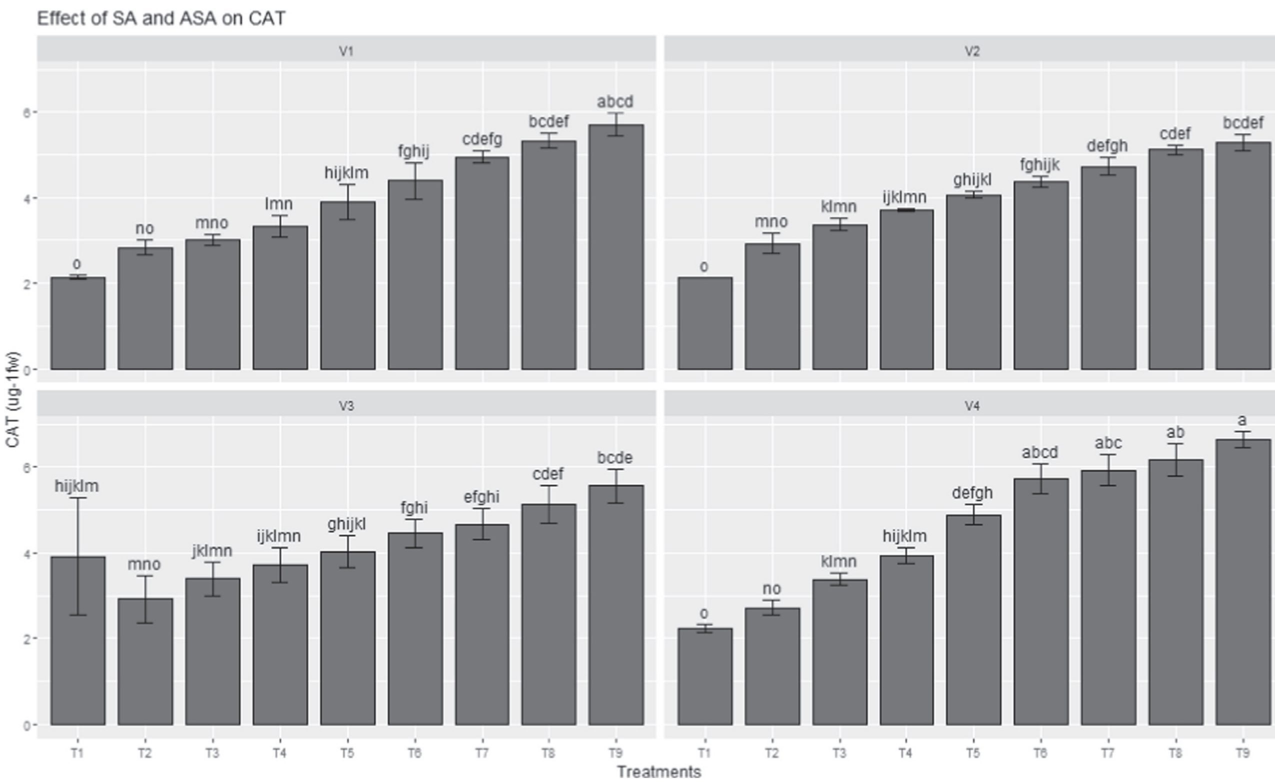
Capsaicinoids are non-volatile compounds like capsaicin, nor capsaicin dihydrocapsaicin, homodihydrocapsaicin and nor-dihydrocapsaicin, (Al Othman et al., 2011) which give chillies its hot taste, (Orobiyi et al., 2015) and are international sought by pharmaceutical industries for their therapeutic characteristics (Reddy and Sasikala, 2013).

Ascorbic acid (AA) can play an important role to stress resistance in plants and enhance plant growth which help into increase yield. (Zhou et al., 2016). It increases water retention ability of plants during drought stress (Noman et al., 2015). Another investigation revealed that AA is an important tool to control various

* Corresponding author at: School of Landscape Architecture and Ornamental Horticulture, Beijing Forestry University, PR China.
E-mail address: gaoyk@bjfu.edu.cn (G. yike).

https://doi.org/10.1016/j.jksus.2023.102660
1018-3647/

1)



- **Pairwise comparison under LSD test, lettering comparison of treatments within varieties and varieties between treatments analyzed and compare.**

Fig. 1. CAT ug[1] fw (1); POD ug[1] fw (2); SOD ug[1] fw (3); Proline (%) (4); Carbohydrates (%) (5); Protein (%) (6); Fat (%) (7); Ash(%) (8) Fiber (%) (9) Yield (g) (10) of four hybrid varieties of chillies with the foliar application of ascorbic acid, salicylic acid and their combination with different concentration T1 (Control) T2 (SA 1mM), T3 (SA 2mM), T4 (AA 1mM), T5 (AA 2mM), T6 (SA 1mM + AA 1mM), T7 (SA 1mM + AA 2mM), T8 (SA 2mM + AA 1mM) and T9 (SA 2mM + AA 2Mm) V1=SV8233-HD (V2), SV5232-HY (V3), SV8883-HA (V4) and Golden heart.

steps involved from germination of seeds like plant growth and development, response to biotic and abiotic stress stimuli till the plant maturity accompanied by yield enhancement (Latif et al., 2016). Amongst these, ascorbic acid (AA) is a critical component that influences the growth, development, and quality of cut flowers. It behaves as a co-factor, changing phyto-hormone-mediated signaling involved in the transition of the plant from the vegetative to the reproductive phase, involving floral initiation, growth, and, eventually, floral drop (Ghadimian and Danaei, 2020). Foliar application of ascorbic acid promotes the nutritional content of sweet pepper and greatly improves the quality and growth of Italian cypress (Zahid et al., 2021).

Salicylic acid is considered a signaling molecule for production of phenolic compounds and natural plant growth regulator or phyto-hormone. The physiochemical and metabolic mechanisms in plants are regulated by synthesis of Salicylic Acid by independent and dependent pathways in plants (Dempsey and Klessig, 2017) and (Jayakannan et al., 2015). Salicylic acid has positive influence on flower production, electrolyte movements in plants, chlorophyll enhancement and rate of photosynthesis. (Sahu, 2013; Souri and Tohidloo, 2019). Exogenous salicylic acid treatment enhanced vegetable production by minimizing stress-induced growth reduction (Khan et al., 2015). Exogenous use of Salicylic acid (SA) enhanced and demonstrated successful results in di-cotyledons such as heat tolerance, chilling, and salt stress (Basit et al., 2018). According to Abdi and Karami (2020), SA significantly increases germination and biomass accumulation.

Climate change is opposing serious productive challenges to global agriculture. Chillies are very much sensitive to abiotic stresses like heat and water scarcity. These abiotic factor considerably reduce chillies yield and profitability. Exogenous application of various growth regulators like salicylic acid and ascorbic acid has been subject extensive to cope with adverse effects of drought, heat and salinity in crops. Positive effects of growth hormones have already been reported in wheat, tomato and roses (Zahid et al., 2021; El-Hawary et al., 2023).

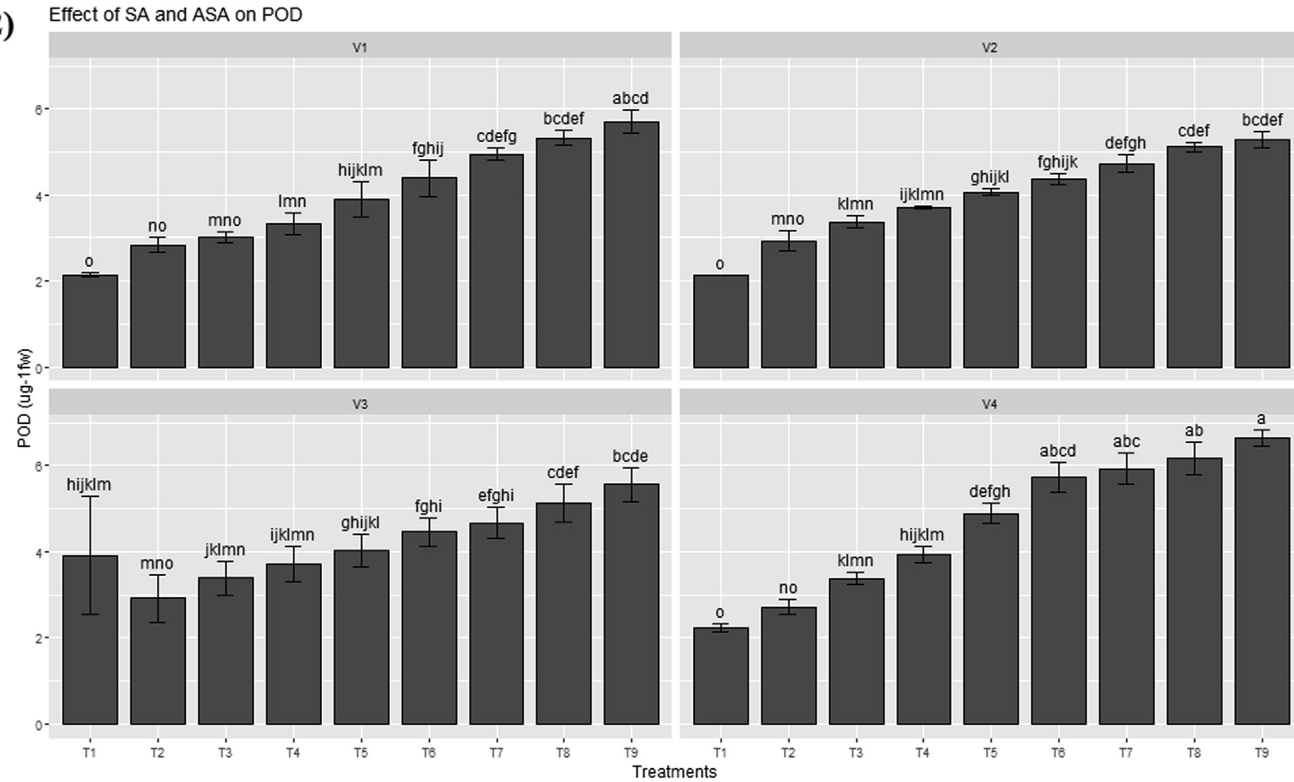
Moreover, foliar application of these growth hormones also improves crop health and productivity. However, study related to response of chillies to foliar application of SA and ASA are limited. Hence this study was designed to investigate the beneficial effect of SA and ASA on growth and development of chillies.

2. Material and method

2.1. Plant material

The study was conducted at Vegetable Research Institute (VRI), Ayub Agricultural Research Institute Faisalabad under field conditions in Pakistan in 2020–2021. Vigorous and uniform seedlings were transplanted into the open field under tunnel with a plastic sheet. We maintained the distance of 75 cm between one row to the other and plant to plant distance is 45 cm, 40 days after sowing (DAS). The experiment was carried out without the application of insecticides and fungicides. Weeds were kept under control by

2)



3)

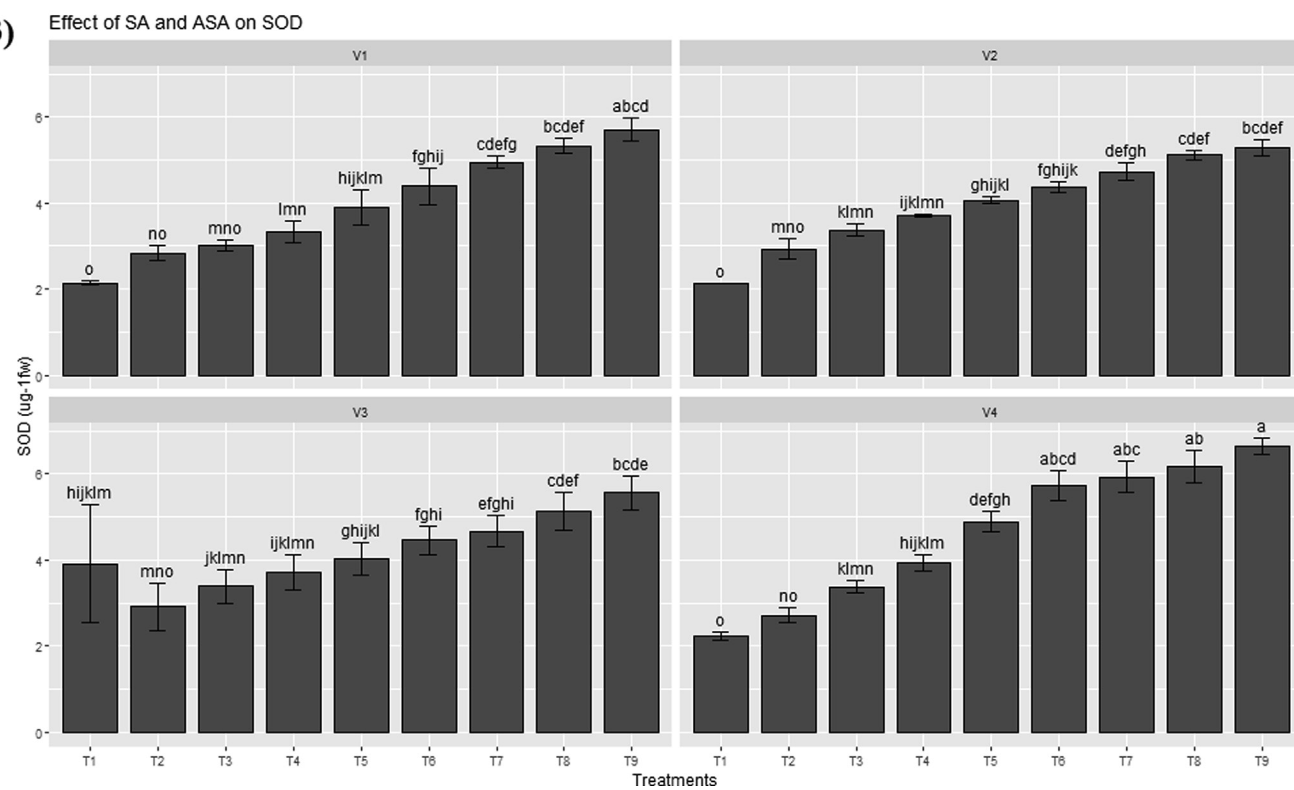
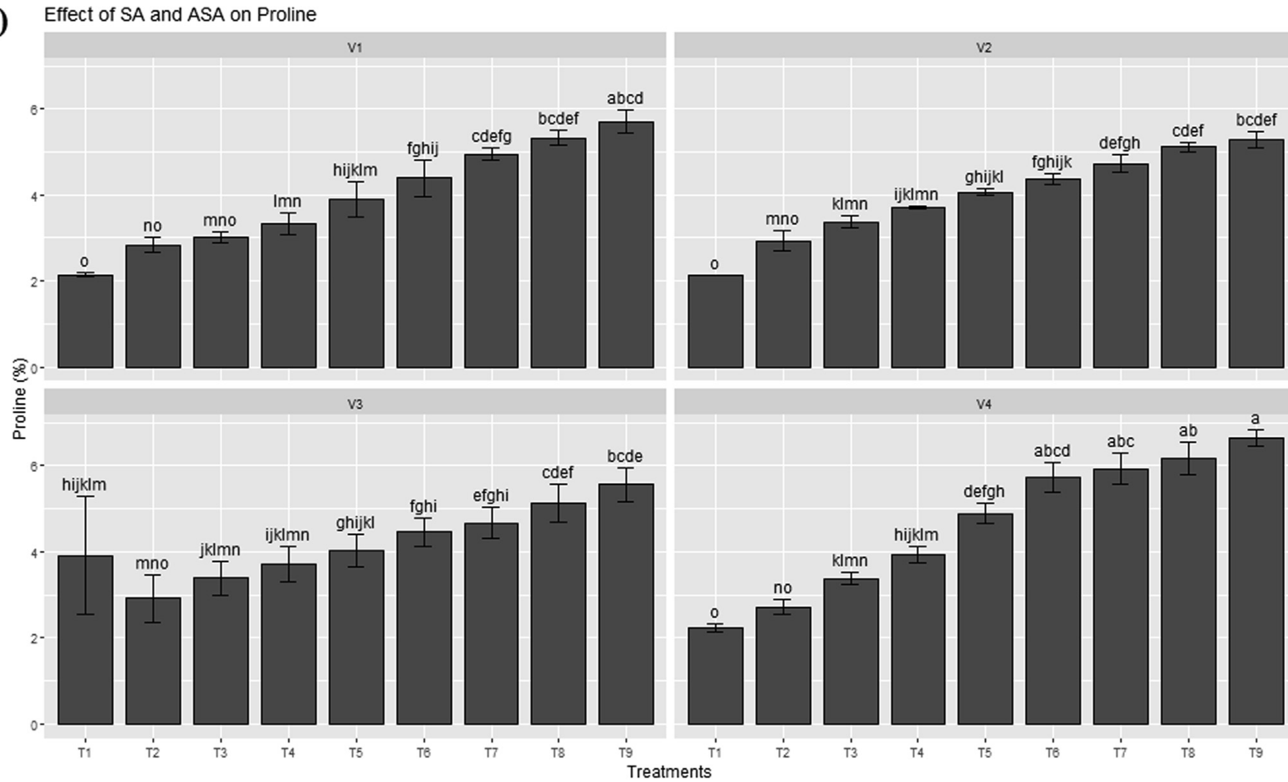


Fig. 1 (*continued*)

hand removing them. All the plots received regular and even cultural practices. There were 3 replicates per treatment and 10 plants per replicates. Foliar application of salicylic acid, ascorbic acid and their combination were applied 25 days after transplanting with 10 day intervals at the stages of planting, initiation of flowering and fruiting. The different levels of salicylic acid and ascorbic acid such as T1 (Control) T2 (SA 1 mM), T3 (SA 2 mM), T4 (AA 1 mM), T5 (AA 2 mM), T6 (SA 1 mM + AA 1 mM), T7 (SA 1 mM + AA 2 mM), T8

4)



5)

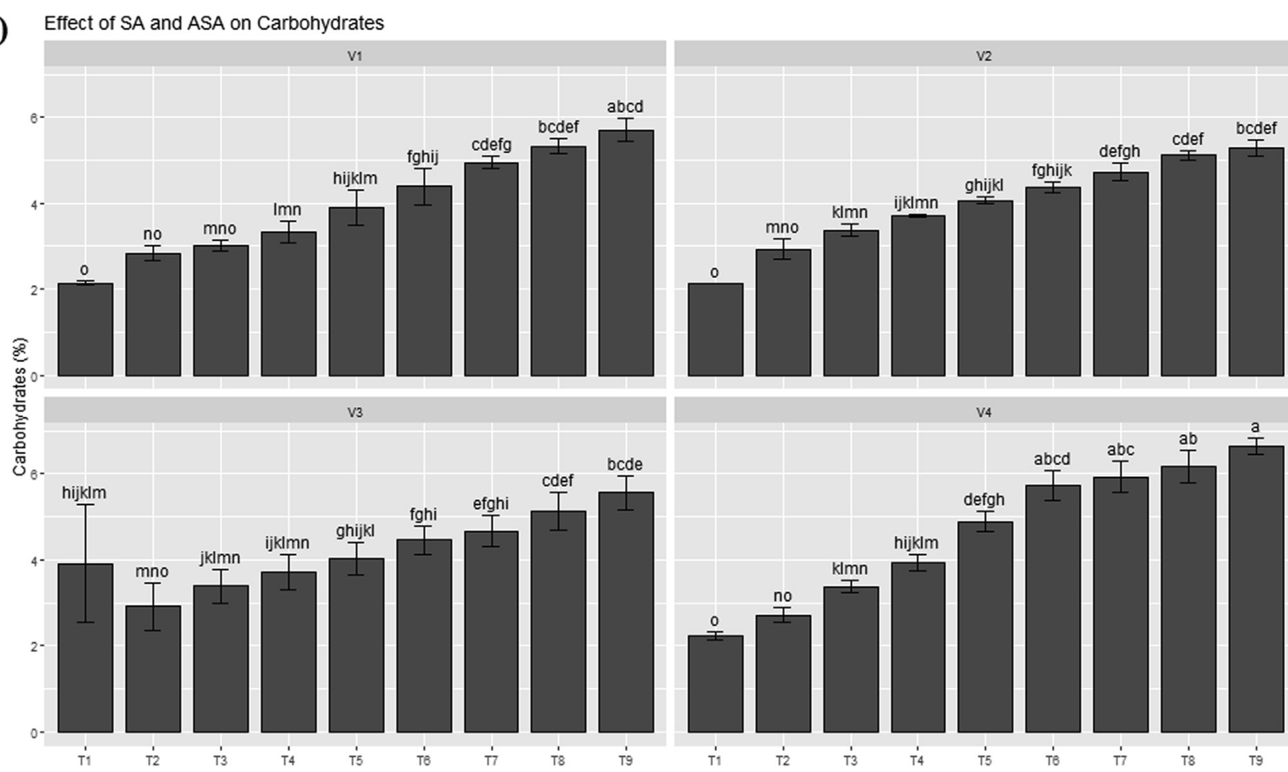
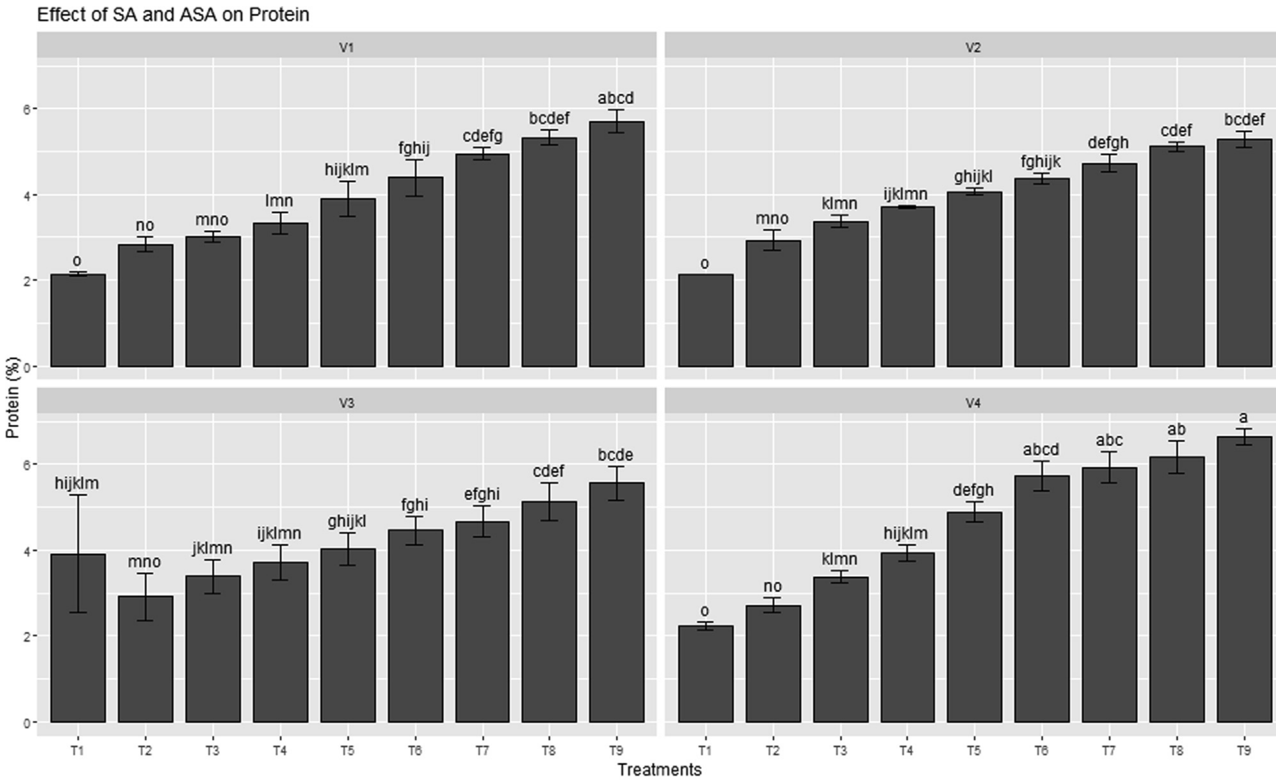


Fig. 1 (*continued*)

6)



7)

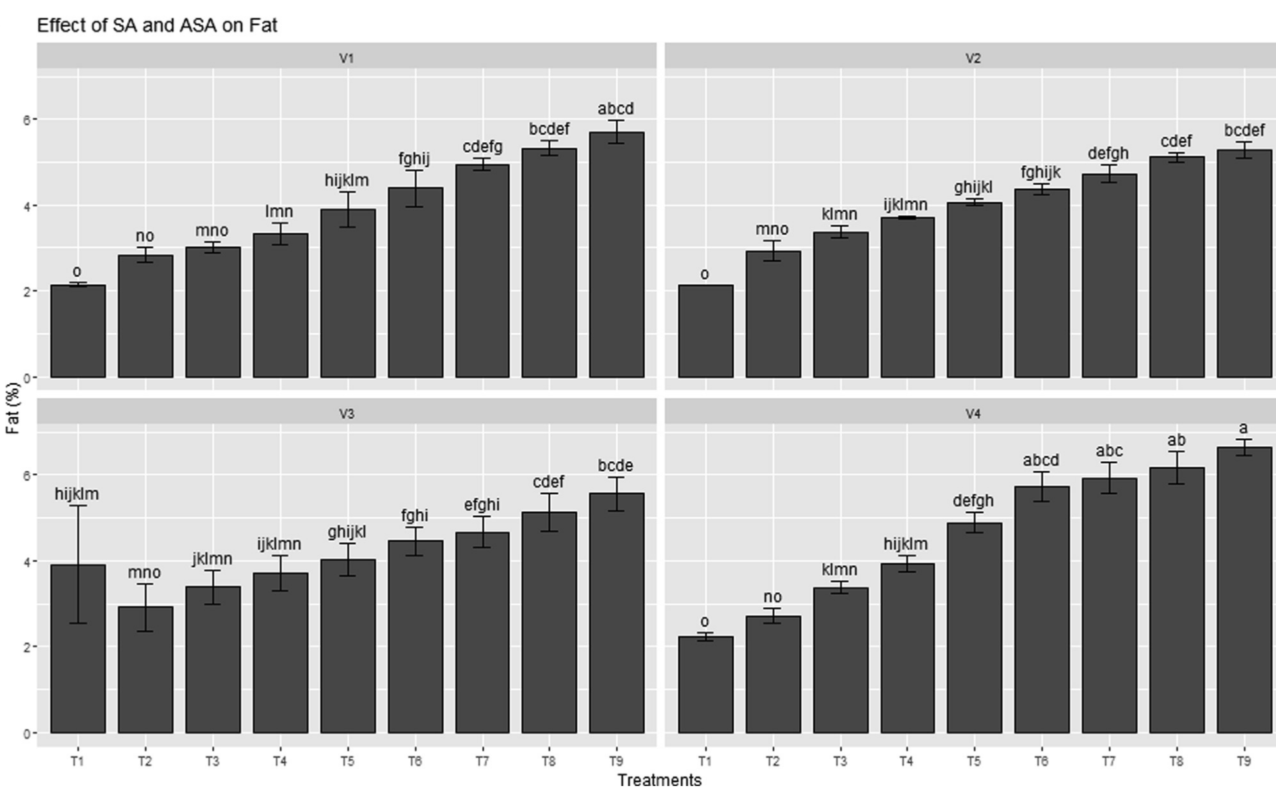
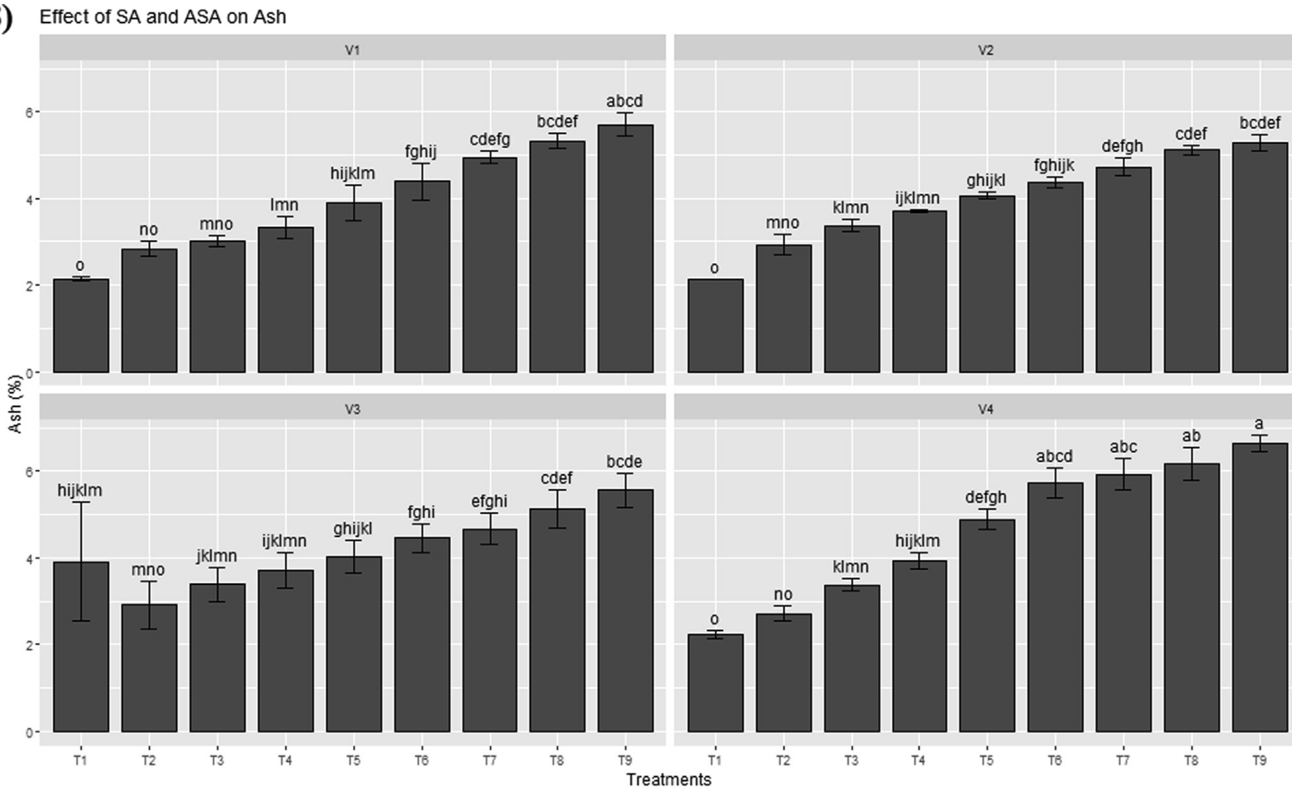


Fig. 1 (*continued*)

8)



9)

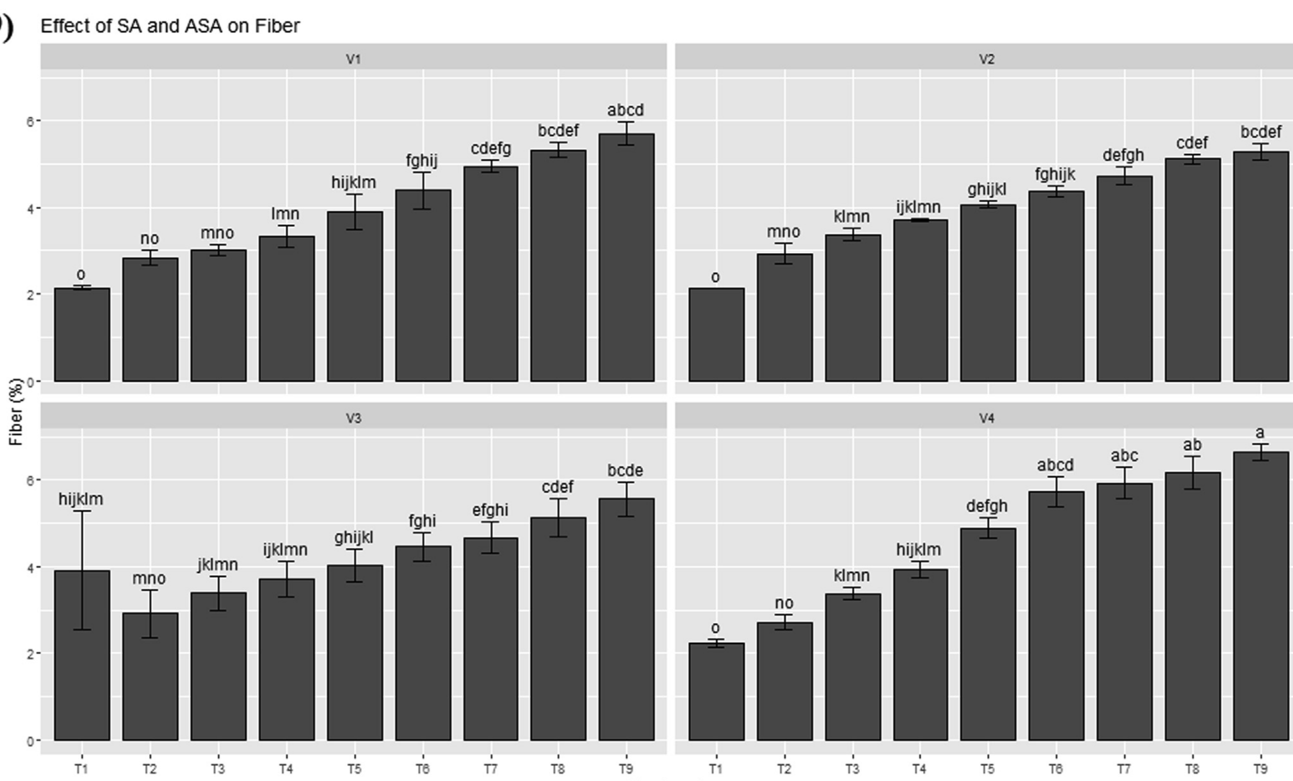


Fig. 1 (*continued*)

10)

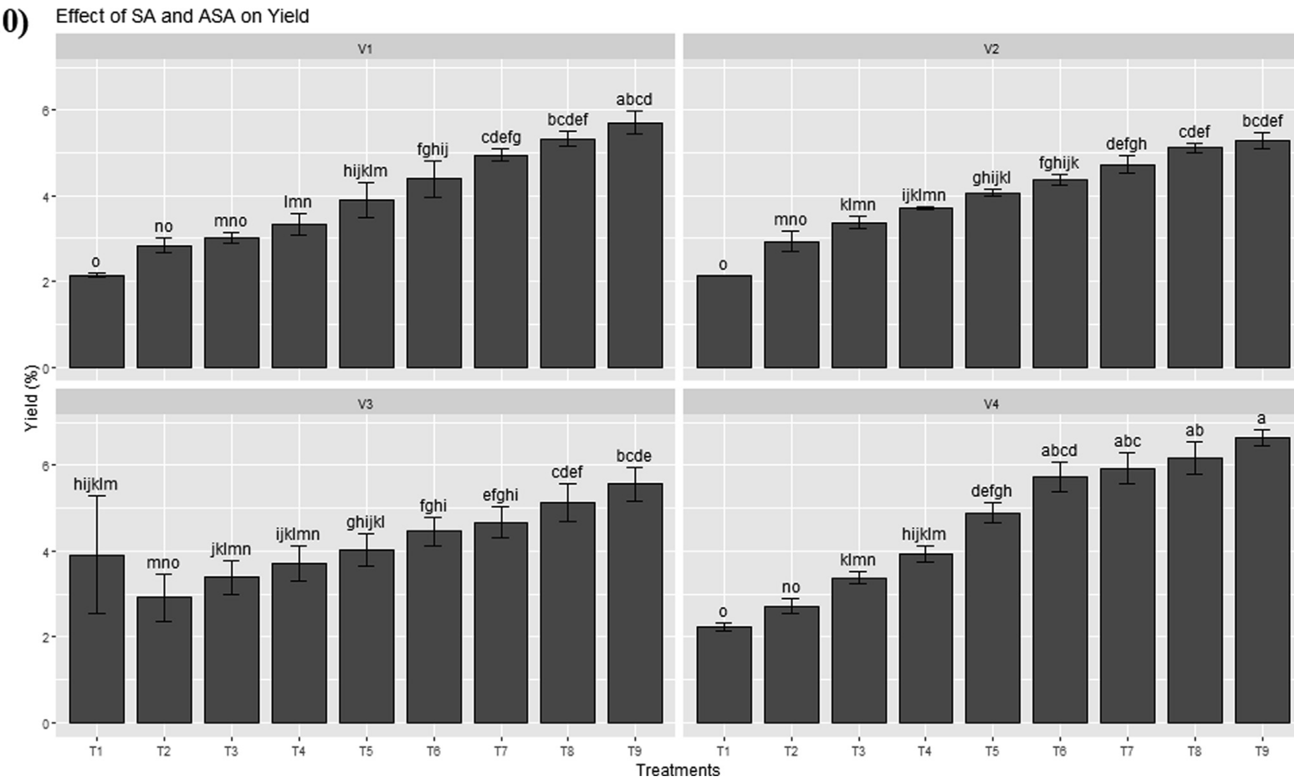


Fig. 1 (*continued*)

(SA 2 mM + AA 1 mM) and T9 (SA 2 mM + AA 2 mM) used in four hybrid varieties V1 = SV8233-HD (V2), SV5232-HY (V3), SV8883-HA (V4) and Golden Heart.

2.2. Vegetative growth parameter

Vegetative growth parameters i.e., Plant height (cm), Fruit weight (g), yield (g) root weight (g) shoot weight (g), Fruit width (mm) fruit length (mm), and pericarp thickness (mm) were estimated from five guarded plants in every treatment and their means was calculated.

2.3. Estimation of CAT activity

A reaction mixture containing 15 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2 was used to estimate Catalase Enzyme Activity (CAT) using procedure described by Dhindsa et al. (1981). The absorbance fluctuations were taken at 240 nm after 40 s. The amount of enzyme activity equivalent to amount of enzyme required to reduce absorption from reaction mixture at 240 nm within one minute was equivalent to one unit of CAT.

2.4. Estimation of SOD activity

1000 ll of enzymatic extracts with 2.50 ml of 55.0 M methionine, 100 mM of phosphate buffer (pH 7.8), 300 ml nitrobluetetrasolium 0.75 mM and 60 ll of 0.1 mM riboflavin were kept in a test tube and incubated for 10 min in fluorescence light (40 lmol m-2 s-1) using method performed by the Xu et al. (2008). The absorbing solution read to 560 nm by a UV/visible spectrometer. One unit of SOD enzyme was determined as the amount of enzyme that inhibits 50% of photo-reduction NBT.

2.5. Estimation of POD activity

POD activity was calculated from 50 ml of enzyme extract placed into reaction mixture, 0.4% H2O2, potassium phosphate buffer to pH 6.1 and 1% guaiacol using by the Zhou and Leul (1998) method with absorbance at 470 nm.

2.6. Estimation of proline amount

A step wise procedure developed by Bates et al. (1973) was adopted to find out the amount of proline. Firstly, a homogenized leave sample of 0.2 g obtained through centrifugation at 18,000 g for 15 min was obtained. 2 ml of leave sample taken in a test tube was mixed with 2 ml glacial acetic acid and freshly prepared acid nin-hydrin solution (1.25 g nin-hydrin dissolved in 20 ml 6 m orthophosphoric acid and 30 ml glacial acetic acid). Heating of test tube solution at 100 °C for 1 h and cooled at 25 °C. Then, 4 ml of toluene were poured into the contents of the test tube in phase separation stage placing test tubes at vertical position for 10 min. The absorbance reading was observed at 520 nm and content of proline was amounted in lg g^{-1}on weight basis.

2.7. Estimation of proximate

Protein, carbohydrates, fat, crude fiber and ash content were measured by AOAC (2002).

2.8. Statistical analysis

The data recorded was subjected to analysis of variance following through R Studio (4.2.2). All graphical presentation by R Language (ggplot2) Scheme.

Result and Discussion.

3. Results

3.1. Plant height (cm)

Analysis of variance showed significant differences between four varieties of chillies subjected to various levels of salicylic acid and ascorbic acid and their combination. However significant differences were observed in V4 plant height growth in response to different applications of salicylic acid and ascorbic acid. Out of nine salicylic acids, ascorbic acid and their combination, maximum plant growth was given by T9 (80.3 cm) whereas minimum plant growth in 33 cm were observed in T9 and T1 in chilli varieties. In variety V3 at the treatment T9 (58 cm) was observed in chillies i.e. minimum plant height growth was observed in T1 (34 cm). Moreover, maximum plant height at T9 (69 cm) less growth of plant height was observed (45 cm) in variety V2. Whereas the highest plant height in T9 (51.6 cm) and lowest plant height (33 cm) in variety of V1. It is depicted that different levels of salicylic acid, ascorbic acid and their combination strains significantly affected the plant height in chillies (Table 1).

3.2. Root weight (g) and shoot weight (g)

The data regarding the chillies plant growth responses to ascorbic and salicylic acid presented in Table 1 revealed that foliar of AA and SA applications significantly improved plant dry weight (g) and shoot weight (g) compared to control plants. The maximum value of shoot weight observed in T9 (161 g) and the minimum value of (71 g) in variety V4. The highest shoot weight of T9 (123.3 g) and the lowest weight in T1 (60.33 g) in variety V3. The shoot weight greater in T9 (141.0 g) and lower in control treatment of V2 as followed by V1 variety of chillies. Whereas the highest value of root weight in treatment of T9 (47.16 g) as compared to T1 (20.10 g) variety of V4. In variety V3 treatment T9 showed root weight (32.43 g) and minimum value in T1 (18.03 g). The values of root weight T9 (39.10 g) and T1 (16.10 g) in V2 as the same the highest value T9 (36.33 g) and lowest value T1 (13.46). The highest values of growth parameters were recorded, ascorbic and salicylic acid applied together with all concentrations as foliar with respect either individual or control plants.

3.3. Fruit width and fruit length (mm)

Different concentrations of SA and AA on different chilli varieties resulted in significant differences on fruit length constituting range of fruit length from 96.6 mm to 81.0 mm.The maximum fruit length (96.6 mm) was measured in T9 treatment for variety V3 followed by, 99.0 mm for V2 and (95.0 mm) for V1 respectively as compared to control. Similarly, greater fruit width(30.13 mm) was observed in treatment T9 and lowest (17.10 mm) in T1 for variety V4 which was statistically similar with variety V3, V2 and V1 of chillies.

Table 1

Varieties	Foliar application	Plant height (cm)	Shoot weight (g)	Root weight (g)	Fruit width (mm)	Fruit Length (mm)	Pericarp thickness (mm)	Fruit weight (g)
V1	T1	33.0 ± 01.0u	66.0 ± 1.0k	13.46 ± 0.3s	13.43 ± 1.5op	57.0 ± 1.0uv	1.50 ± 0.1s	6.1 ± 0.2k
	T2	36.66 ± 0.5q	73.0 ± 1.0*m*	17.73 ± 0.4q	18.10 ± 1.0lm	61.0 ± 1.0tu	1.68 ± 0.1k	7.0 ± 0.6*m*
	T3	38.0 ± 1.0no	80.6 ± 1.5j	19.16 ± 0.2op	19.26 ± 1.2kl	69.0 ± 1.0qrs	1.69 ± 0.01j	7.9 ± 0.9j
	T4	42.00 ± 1.0kl	86.0 ± 1.0f	20.80 ± 0.3*n*	21.13 ± 0.9ij	74.0 ± 1.0opq	1.73 ± 0.01*h*	9.3 ± 0.4f
	T5	43.66 ± 0.5qr	91.6 ± 1.5e	23.23 ± 0.4lm	22.43 ± 0.5hi	81.0 ± 1.0lmn	1.84 ± 0.01d	11.0 ± 1.3e
	T6	45.66 ± 0.5q	96.0 ± 1.0c	27.70 ± 0.5k	23.10 ± 1.0gh	87.33 ± 1.5ghi	1.89 ± 0.01c	13.0 ± 1.0c
	T7	47.66 ± 0.5p	103.0 ± 2.6b	29.76 ± 0.4hi	24.20 ± 0.8fg	91.0 ± 1.0efg	1.92 ± 0.01c	14.6 ± 0.8b
	T8	49.66 ± 0.5o	113.0 ± 2.6a	32.36 ± 0.5g	25.56 ± 0.7ef	93.0 ± 1.0cde	1.99 ± 0.01b	15.7 ± 0.6a
	T9	51.66 ± 0.5mn	122.6 ± 2.5e	36.33 ± 0.5ef	26.20 ± 1.0de	95.0 ± 1.0cde	2.10 ± 0.1a	16.6 ± 0.6a
V2	T1	45.00 ± 1.0qr	81.6 ± 1.5j	16.10 ± 0.9r	12.26 ± 0.9p	49.0 ± 1.0bct	1.51 ± 0.02f	7.9 ± 1.0e
	T2	48.00 ± 1.0p	88.0 ± 1.0i	20.13 ± 1.0no	16.26 ± 0.9*m*	53.0 ± 1.0w	1.54 ± 0.01p	8.3 ± 0.3j
	T3	52.00 ± 1.0*m*	95.6 ± 1.5g	24.26 ± 1.0l	20.23 ± 0.8jk	59.0 ± 1.0vw	1.60 ± 0.02o	8.8 ± 0.3i
	T4	57.00 ± 1.0ij	101.6 ± 1.5f	28.30 ± 1.1jk	21.23 ± 0.9ij	63.3 ± 1.0uv	1.65 ± 0.01*l*	9.3 ± 0.3g
	T5	59.00 ± 1.0gh	121.6 ± 1.5e	30.13 ± 1.0h	22.36 ± 0.9hi	67.0 ± 1.0stu	1.703 ± 0.01j	9.9 ± 0.3f
	T6	62.00 ± 1.0f	126.0 ± 1.0e	33.10 ± 1.0g	23.20 ± 1.0gh	71.0 ± 1.0rst	1.75 ± 0.01*h*	10.5 ± 0.3e
	T7	64.33 ± 0.5e	131.0 ± 1.0e	35.10 ± 1.0f	24.40 ± 0.6fg	74.0 ± 1.0pqr	1.79 ± 0.01f	10.8 ± 0.2e
	T8	67.0 ± 1.0d	136.0 ± 1.0d	37.46 ± 1.5e	25.10 ± 1.0ef	77.0 ± 1.0opq	1.83 ± 0.01d	10.9 ± 0.5e
	T9	69.0 ± 1.0c	141.0 ± 1.0f	39.10 ± 1.0d	26.10 ± 1.0de	99.0 ± 1.0nop	1.85 ± 0.01d	11.6 ± 0.4a
V3	T1	34.0 ± 1.0w	60.33 ± 1.5a	18.26 ± 1.0pq	12.40 ± 1.2p	70.0 ± 1.0pqr	1.48 ± 0.02s	5.3 ± 1.0d
	T2	38.0 ± 1.0v	68.0 ± 2.0d	19.36 ± 1.0op	14.00 ± 0.7o	75.0 ± 1.0opq	1.53 ± 0.02r	5.9 ± 1.0p
	T3	43.0 ± 1.0st	70.3 ± 1.5p	20.20 ± 1.0no	14.66 ± 0.4o	79.0 ± 1.0mno	1.58 ± 0.02p	6.2 ± 1.0o
	T4	44.6 ± 1.5qr	80.0 ± 2.0o	22.50 ± 0.7*m*	16.33 ± 0.9*n*	83.0 ± 1.0jkl	1.63 ± 0.02*m*	6.5 ± 1.0*m*
	T5	48.0 ± 1.0t	97.3 ± 2.5*m*	28.26 ± 1.1jk	17.23 ± 0.9mn	86.0 ± 1.0hij	1.68 ± 0.02k	7.0 ± 1.0l
	T6	52.0 ± 1.0o	108.0 ± 2.0*n*	28.46 ± 0.6ijk	18.20 ± 0.9lm	89.0 ± 1.0fgh	1.73 ± 0.02h	7.5 ± 1.0k
	T7	54.0 ± 1.0*m*	112.0 ± 2.0k	29.23 ± 0.9hij	19.20 ± 0.9kl	92.6 ± 0.5def	1.79 ± 0.03f	8.0 ± 1.0j
	T8	56.0 ± 1.0jk	117.0 ± 2.0j	30.13 ± 1.0h	22.20 ± 0.9hi	93.6 ± 0.5cdf	1.84 ± 0.01e	8.4 ± 0.2h
	T9	58.0 ± 1.0hi	123.3 ± 3.0h	32.43 ± 0.6g	23.20 ± 1.0gh	96.6 ± 0.6cde	1.89 ± 0.02c	8.4 ± ± 0.3h
V4	T1	41.0 ± 1.0u	71.0 ± 1.0p	20.10 ± 1.0no	17.10 ± 1.0mn	81.0 ± 1.0lno	1.62 ± 0.01*n*	5.5 ± 0.1p
	T2	45.3 ± 1.5q	79.0 ± 1.0l	24.46 ± 0.9l	19.13 ± 0.9kl	84.6 ± 1.0jkl	1.65 ± 0.01*l*	6.6 ± 0.1l
	T3	50.33 ± 1.5no	86.0 ± 1.0k	29.23 ± 0.9hij	21.16 ± 0.9ij	89.0 ± 1.0fgh	1.69 ± 0.02j	7.0 ± 0.1k
	T4	55.3 ± 1.5kl	95.0 ± 1.0j	32.16 ± 0.9g	23.10 ± 1.0gh	85.3 ± 1.0jk	1.73 ± 0.01*h*	7.4 ± 0.1j
	T5	60.3 ± 1.5g	111.0 ± 1.0j	36.23 ± 1.0ef	25.16 ± 1.0ef	90.3 ± 1.0efg	1.75 ± 0.01*h*	7.9 ± 0.1j
	T6	65.3 ± 1.5e	131.0 ± 1.0h	39.26 ± 0.9d	27.16 ± 0.9cd	95.3 ± 1.0cde	1.78 ± 0.01g	8.4 ± 0.1h
	T7	70.3 ± 1.5c	146.0 ± 1.0g	42.16 ± 1.0c	28.10 ± 1.0bc	100.3 ± 1.0bc	1.82 ± 0.01e	8.7 ± 0.2g
	T8	75.3 ± 1.5b	151.0 ± 1.0f	45.23 ± 1.0b	29.13 ± 1.0ab	105.3 ± 0.5ab	1.87 ± 0.01c	9.2 ± 0.2f
	T9	80.3 ± 1.5a	161.0 ± 1.0e	47.16 ± 0.9a	30.13 ± 1.0a	110.3 ± 1.0a	1.99 ± 0.17b	10.2 ± 0.1e

Mean value (n = 3) in the same column with the same following letter do not significantly differ ($p < 0.05$). (T1 (Control), T2 (SA 1mM), T3 (SA 2mM), T4 (AA 1mM), T5 (AA 2mM), T6 (SA 1mM + AA 1mM), T7 (SA 1mM + AA 2mM), T8 (SA 2mM + AA 1mM) and T9 (SA 2mM + AA 2Mm) of V1=SV8233-HD (G1), SV5232-HY (G2), SV8883-HA (G3) and Golden heart.

3.4. Peri-carp thickness (mm)

The maximum value of *peri*-carp thickness in T9 (1.99 mm) and minimum (1.62 mm) for variety V4 was calculated. Moreover, in variety V3 the value was measured in T9 (1.89 mm) and T1 (1.48 mm). The greater value of *peri*-carp thickness of V2, V1 was showed (1.85 mm) and (2.10 mm) respectively as compared to control.

3.5. Fruit weight (g)

Significant impacts of SA and AA was estimated for fruit weight and maximum magnitude of the trait under study was observed in T9 (10.2 g) for V4 followed by (8.4 g) in V3. However, the highest fruit weight (11.6 g), (16.6 g) was recorded in treatment T9 in V2 and V1 plants as compared to T1 treatment.

3.6. Fat (%)

Fat percentage was decreased significantly through foliar spray of SA and AA with the greatest value of T1 (2.7%) in variety of V4. The fat (2.5%) of T1 variety V3 was recorded in plants. However, the lowest fat concentration (1.3%) at T9 in V3 and V1 was recorded. The amount of fat percent present in treatment T9 (1.6%) in V2 as compared to T1 treatment.

3.7. Ash (%)

A significant mean differences for ash percentage was estimated for different concentrations of foliar spray of SA and AA. The greatest value of T9 (9.2%), (8.2%) in varieties of V4 and V3 was recorded in plants. However, the greatest value of ash content (7.8%), (7.6%) was recorded in treatment T9 in V2 and V1 plants as compared to T1 treatment.

3.8. Protein (%)

Highest crude protein was found in T9 (23.48%) of V1 followed by (8.8 %) in V4. Lowest protein was recorded in V3, V2 in treatments in T9 (8.4 %) and (7.9%).

3.9. Carbohydrate (%)

The present results revealed high carbohydrate levels. Highest carbohydrates were found in T9 (57.27 %), followed by (52.49%) in varieties of V4 and V3 as compared to control. In varieties V2 and V1 (51.26 %), (48.87%) was measured in treatment T9 respectively.

4. Fiber (%)

The investigation showed that highest value (34.12%) fiber was depicted in treatment T9 in both varieties of V3, V1. The fiber content of treatment T9 (32.78%) in V2, while the T9 (31.78%) was found in variety of V4 as compared to control.

4.1. Proline (%)

The data regarding proline content at various SA and AA foliar application revealed the mean differences for treatments and chilli varieties. Maximum proline contents of 19.1% was found in V2 followed by V1 having value of 18.5%. Furthermore, in treatment T9 maximum value of proline (%) was observed in V3 (17.65%) and V4 (17.85%)respectively.

4.2. Antioxidant enzymatic activity. SOD (ug-1Fw), POD (ug-1Fw), CAT (ug-1Fw).

The maximum value of SOD influenced by the SA and AA application. The results showed that maximum SOD content present in treatment (T9) (1.40 ug-1Fw) V4 (1.39 ug-1Fw) V3. The highest value of SOD in T9 (1.28 ug-1Fw), (1.12 ug-1Fw) V2 and V1 respectively. The maximum POD contents were recorded in treatments T9 (17.9 ug-1Fw), (17.75 ug-1Fw), (17.65 ug-1Fw) and (17.30 ug-1Fw) of varieties V4, V2, V3 and V1 respectively. The highest value of CAT contents in the treatment of T9 (6.15 ug-1Fw) variety of V4. Least value of CAT contents in pepper plant was seen in (5.70 ug-1Fw), (5.53 ug-1Fw) and (5.19 ug-1Fw) varieties of V1, V3, V4 respectively.

4.3. Yield (g)

The yield of pepper varieties was estimated by the foliar application of SA, AA and their combination. The largest yield of T9 (217.66 g) in variety V3 as compared to T1 treatment. The yield was measured (215.33 g) in V4 of T9. The highest value of yield estimated in (208.6 g) and (205.3 g) V1 and V2 as compared to control. It cleared that ascorbic acid and salicylic acid at their different levels of applications significantly increased all varieties yield compared to control.

5. Discussion

Morphological attributes.

5.1. Plant height (cm)

The foliar application of SA mitigated the growth reducing effects constituted due to various abiotic stresses thus, improved plant height, number of leaves plant per, chlorophyll content, fruit quality and yield as compared to control (Table 1). Such inferences were in accordance with the result findings of Zaghlool et al. (2006) who concluded additive association with plant growth and fruit yield parameters with the application of SA. Moreover, in addition to increased plant growth parameters like plant height, stem diameter, number of branches, number of leaves, root length as well fresh and dry weight the nitrogen, phosphorus, potassium uptake percentages were also increased with increasing concentrations of ascorbic acid as foliar application (Mazhar et al., 2011).

5.2. Yield

The impact of different formulation of SA significantly improved plant growth and becomes cause of increase in yield of the plant. Results showed that foliar spraying of ascorbic acid at 100 mg/l increased yield and improved plant characteristics such as, number of total and folded leaves, fresh weight of total and folded leaves, leaf area per plant, plant fresh and dry weight and leaves dry matter and total soluble solid (Jerry et al., 2011; Nafeesa et al., 2019).

5.3. Fruit width (mm) and fruit length (mm)

The foliar applications of SA indicated positive influence on some fruit characteristics, earliness, yield, chlorophyll contents in leaves and plant growth. However, SA treatments had no effect on pH, AA and TA of tomato (Yildrim and Dursun, 2009).

Biochemical attributes

5.4. Carbohydrate

Many previous observations, while for different crops, demonstrated that the application of SA altered the growth, photosynthesis and carbohydrates metabolism of maize and sugar beet. (Khaled and colleagues, 2020). Carbohydrates contents have positive relation with the foliar application of ascorbic acid due to reducing effects of plant stress on plant growth. The massive increase in total contents of carbohydrates due to foliar application of ascorbic acid in our case was resembled with Mazhar et al. (2011).

5.5. Proline

Foliar application of salicylic acid helpful to increase the proline contents in linseed (Bakry et al., 2012). The increase in proline content to combat the reducing effects of abiotic plant stresses matched the results with Nazi et al., 2016 who conducted experiment on cucumber in arid conditions and found Ascorbic acid stimulation in the chlorophyll *a*, proline, relative water content and Ci concentration).

5.6. Fat

Salicylic acid increased the fatty acid content under the drought stress in purslane plant. However, our outcomes contrast to the Saheri et al (2020). According to Aml et al. (2011) exogenous treatment of ascorbic acid boosted all fatty acids in the sunflower crop.

5.7. Fiber and ash

Out results correlates with the Meena et al. (2012) by the foliar spray of ascorbic acid and salicylic acid increase the fiber and ash contents in Aswagandha (Withania somnifera L.Dunal).

5.8. Protein

Highest values of protein content were noted with the treatment application of Acetyl Salicylic Acid at concentrations 0.5 mM in grain. Our results correlate with the Matysiak et al. (2020). Stating that spray of ascorbic acid increases the protein in maize at the concentration of 8 g /l. Our results of protein are similar to Lamiaa et al. (2020).

5.9. Enzymatic Activities:

In broad beans, foliar application of SA enhanced the activities of antioxidants enzymes and all growth parameters (Azooz et al., 2011). The use of AA along with 0.03% Zn combination enhanced photosynthetic pigmentation, vegetative growth, ion uptake, antioxidant activity in lettuce (Noreen et al., 2021).

6. Conclusion

Chillies productivity is a product of multitude of metabolic processes and their interaction to environment. Unfavorable environment like high/low temperature, drought and salinity etc. disrupts plant's physiology and effects yield. Foliar application of Salicylic Acid and Ascorbic Acid significantly enhanced activity of antioxidant enzymes (SOD, POD & CAT) and proline contents in chillies which are amongst the first line of defense against stresses. Moreover, growth attributes of chillies are also improved significantly. The results of following study suggest that exogenous application of Salicylic Acid and Ascorbic Acid at optimum dose enable chilli plants to cope in negative environment factors better which translates into increased yield and profitability.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are thankful to the Chinese government scholarship council, Beijing Forestry University, Beijing China for providing us an environment of learning. This work was funded by Researchers Supporting Project number (RSP2023R414), King Saud University, Riyadh, Saudi Arabia.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102660.

References

Abdi, G., Karami, L., 2020. Salicylic acid effects on some physiochemical properties and secondary metabolite accumulation in peppermint (*Mentha piperita* L.) under water deficit stress. Adv. Hortic. Sci. 34, 81–91.
Al Othman, Z.A., Ahmed, Y.B.H., Habila, M.A., Ghafar, A.A., 2011. Determination of Capsaicin and Dihydrocapsaicin in Capsicum Fruit Samples using High Performance Liquid Chromatography. Molecules. 16, 8919–8929.
Aml, E.A.E., Farouk, S., Abdl El-Ghani, A.H.M., 2011. Evaluation of different seedpriming onseedling growth, yield and quality components in two sunflowers (*Helianthus annuus* L.) cultivars. Trends. Appl. Sci. Res. 9, 977–991.
Amruthraj, N.J., Raj, J.P.P., Lebel, L.A., 2014. Effect of vegetable oil in the solubility of capsaicinoids extracted from *Capsicum Chinense* Bhut Jolokia. Asian J. Pharm. Clin. Res. 7, 48–51.
AOAC, 2002. A Official methods of analysis (17th ed.). Washington, DC: Association of Official Analytical Chemists.
Azooz, M.M., Youssef, A.M., Parvaiz, A., 2011. Evaluation of salicylic acid (SA) application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedlings grown under diluted seawater. Int. J. Plant Physiol. 3, 253–264.
Bakry, B.A., El-Hariri, D.M., Sadak, M.S., El-Bassiouny, H.M.S., 2012. Drought Stress Mitigation by Foliar Application of Salicylic Acid in Two Linseed Varieties Grown Under Newly Reclaimed Sandy Soil. J. Appl. Sci. Res. 8, 3503–3514.
Basit, A., Shah, M.U., Rahman, L.X., Zuo, X., Han, M., Alam, N., Khan, F., Ahmed, I., Khalid, M.A., 2018. Salicylic acid an emerging growth and flower inducing hormone in marigold (*Tagetes* sp. L.). Pure Appl. Biol. 7, 1301–1308.
Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. J. Plant Soil 39, 205–207. https://doi.org/10.1007/BF00018060.
Chigoziri, E., Ekefan, E.J., 2013. Seed borne fungi of Chilli Pepper (Capsicum frutescens) from pepper producing areas of Benue State. Nigeria. Agric. Biol. J. N. Am. 4, 370–374.
Dempsey, D.A., Klessig, D.F., 2017. How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans?. BMC Biol. 15, 23. https://doi.org/10.1186/s12915-017-0364-8.
Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A., 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32, 93–101. https://doi.org/10.1093/jxb/32.1.9.
El-Hawary, M.M., Omnia, S.M.H., Hasanuzzaman, M., 2023. Seed Priming and Foliar Application with Ascorbic Acid and Salicylic Acid Mitigate Salt Stress in Wheat. Agronomy. 13, 493. https://doi.org/10.3390/agronomy13020493.
Ghadimian, S., Danaei, E., 2020. Influences of ascorbic acid and salicylic acid on vase life of cut flowers rose (*Rosa hybrida* cv. black magic). J. Enviot. Agric. Biol. Sci. 2, 1–6.
Jayakannan, M., Bose, J., Babourina, O., Rengel, Z., Shabala, S., 2015. Salicylic acid in plant salinity stress signaling and tolerance. Plant Growth Regul. 76, 25–40. https://doi.org/10.1007/s10725-015-0028-z.
Jerry, A.N., Abdullah, A., Alderawy, K.A., Basrah, E., 2011. Effect of foliar spray of ascorbic acid on yield and quality of lettuce (Lactuca sativa L.) grown in southern Iraq. J. Agric. Sci. 24 (57).
Khaled, A.A.A., Rashed, S.H., Hossain, A., Sabagh, A.E.L., 2020. Yield and quality of two sugar beet (Beta vulgaris L. ssp. vulgaris var. altissima Döll) cultivars are influenced by foliar application of salicylic acid, irrigation timing, and planting density. Acta Agric. Slovenica. 115. https://doi.org/10.14720/aas.2020.115.2.1159.
Khan, M.I., Fatma, M., Per, T.S., Anjum, N.A., Khan, N.A., 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front. Plant. Sci. 6, 1–17.

Lamiaa, M.-F., Anhar, M.A., Alabdulla, A.S., 2020. Effect of foliar spray with ascorbic acid on two maize cultivars grown under saline soil. Int. J. Agric. Stat. Sci. 16, 2073–2078.
Latif, M., Akram, N.A., Ashraf, M., 2016. Regulation of some biochemical attributes in drought-stressed cauliflower (Brassica oleracea L.) by seed pre-treatment with ascorbic acid. J. Horti. Sci. Biotechnol. 91, 129–137.
Matysiak, K., Siatkowski, I., Kierzek, R., Kowalska, J., Krawczyk, R., 2020. Effect of foliar applied acetylsalicilic acid on wheat (*Triticum aestivum* L.) under field conditions. Agronomy. 10, 12.
Mazher, A.A., Zaghloul, S.M., Mahmoud, S.A., Siam, H.S., 2011. Stimulatory effect of kinetin, ascorbic acid and glutamic acid on growth and chemical constituents of *Codiaeum variegatum* L. plants. American-Eurasian J. Agric. Envior. Sciences. 10, 318–323.
Meena, K.C., Birla, A.L., Gontia, A.S., Mishra, U.S., Upadhyay, A., Rao, S., 2012. Biochemical and proximate studies of growth promoters on Aswagandha. JNKVV Res. J. 46, 338–342.
Nafeesa, M., Rashid, H., Ishtiaq, A., Ahsana, M., Aslam, M.A., Ahmad, M., Manzoord, A., 2019. Role of Foliar Application of Salicylic Acid and Cultivars in Chilli (Capsicum frutescens L.) Production in Arid Region of Bahawalpur. J. Hortic. Sci. Technol. 2, 5–9.
Nazi, H., Nudrat, A.A., Ashraf, M., 2016. Impact of Ascorbic acid on growth and some physiological attributes of cucumber (*Cucumis Sativus*) plants under Water-Deficit conditions. Pak. J. Bot. 48, 877–883.
Noman, A., Ali, S., Naheed, F., Ali, Q., Farid, M., Rizwan, M., Irshad, M.K., 2015. Foliar application of ascorbate enhances the physiological and biochemical attributes of maize (Zea mays L.) cultivars under drought stress. Arch. Agron. Soil Sci. 12, 1659–1672.
Noreen, S., Sultan, M., Akhter, M.S., Shah, K., H., Ummara, U., Manzoor, H., Ulfat, M., Alyemeni, M.N., Ahmad, P.,, 2021. Foliar fertigation of ascorbic acid and zinc improves growth, antioxidant enzyme activity and harvest index in barley (*Hordeum vulgare* L.) grown under salt stress. Plant Physiol. Biochem. 158, 244–254.
Orobiyi, A., Sanoussi, F., Gbaguidi, A., Dansi, M., Korie, N., Dansi, A., 2015. Ethnobotanical study and varietal diversity of chili pepper (*Capsicum annuum* L). in Central and Northern Benin (in press).
Rahman, M.S., Al-Rizeiqi, M.H., Guizani, N., Al-Ruzaiqi, M.S., Al-Aamri, A.H., Zainab, S., 2013. Stability of vitamin C in fresh and freeze-dried capsicum stored at different temperatures. J. Food Sci. Technol. 52 (3), 1691–1697.
Reddy, D.M.V.B., Sasikala, P., 2013. Capsaicin and colour extraction from different verities of green and red chilli peppers of Andhrapradesh. Int. J. Advanced Sci. Technical. 2, 554–572.
Saheri, F., Barzin, G., Pishkar, L., Boojar, M.M.A., Babaeekhou, L., 2020. Foliar spray of salicylic acid induces physiological and biochemical changes in purslane (*Portulaca oleracea* L.) under drought stress. Biologia. 75, 2189–2200.
Sahu, G.K., 2013. Salicylic acid: Role in plant physiology and stress tolerance. In Molecular, stress physiology of plants 10.1007/978-81-322-0807-5_9. Springer, India, pp. 217–239.
Souri, M.K., Tohidloo, G., 2019. Effectiveness of different methods of salicylic acid application on growth characteristics of tomato seedlings under salinity. Chem. Biol. Technol. Agric. 6, 26. https://doi.org/10.1186/s40538-019-0169-9.
Xu, P.L., Guo, Y.K., Bai, J.G., Shang, W.X., 2008. Effects of long-term chilling on ultrastructure and antioxidant activity in leaves of two cucumber cultivars under low light. Physiol. Plant 132, 467–478. https://doi.org/10.1111/j.1399-3054.2007. 01036.x.
Yıldırım, E., Dursun, A., 2009. Effect of foliar salicylic acid applications on plant growth and yield of tomato under greenhouse conditions. Acta Hort. 807, 395–400.
Zaghlool, A.M., Mostafa, M.A., Shehata, S.A.M., 2006. Physiological studies on the effect of kinetin and salicylic acid on growth and yield of wheat plant. Annals of Agric. Sci. 51, 41–55.
Zahid, A., Yike, G., Kubik, S., Ramzan, M., Sardar, H., Akram, M.T., Khatana, M.A., Shabbir, S., Alharbi, S.A., Alfarraj, S., Skalicky, M., 2021. Plant growth regulators modulate the growth, physiology, and flower quality in rose (Rosa hybirda). J. King Saud Uni. Sci. 33, 101526.
Zhou, X., Gu, Z., Xu, H., Chen, L., Tao, G., Yu, Y., Li, K., 2016. The effects of exogenous ascorbic acid on the mechanism of physiological and biochemical responses to nitrate uptake in two rice cultivars (Oryza sativa L.) under aluminum stress. Plant Growth Regul. 35, 1013–1024.
Zhou, W., Leul, M., 1998. Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. Plant Growth Regul. 26, 41–47. https://doi.org/10.1023/A:1006004921265.