THE EFFECT OF SALICYLIC ACID ON GROWTH AND BIOCHEMICAL CONSTITUENTS OF *CORIANDRUM SATIVUM* L. UNDER DROUGHT STRESS

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ABSTRACT

The physiological role of exogenously applied phenolic compound salicylic acid (0.5 and 1 mg/L) was studied on the performance of Coriander variety Dilpazir seedling under imposed drought stress at 4 leaf stage for 7 days (moisture content 7% soil dry weight). Seedling weight, leaf relative water content, photosynthetic pigments and essential oil content were significantly decreased by drought stress. In response to drought stress plants of Coriander showed the higher build-up of soluble proteins and phenolic compounds in leaves and roots. The content of phenolic compounds was higher in roots than that of leaves. The higher activity of antioxidants enzymes superoxide dismutase (SOD) and peroxidase (POD) was recorded in the leaves, due to drought stress. Foliar application of salicylic acid (SA) minimized the drought-induced reduction in seedling weight, photosynthetic pigments and essential oil content. The antioxidant enzymes activities and soluble phenolic compounds production were further augmented by SA under drought stress. Higher antioxidant enzymes activity and increased build-up of phenolic compounds under drought stress by SA application seems like a possible mechanism involved behind the SA induced drought stress tolerance of coriander.

Key words: Abiotic stress, phenolic compounds, Superoxide dismutase, peroxidase

INTRODUCTION

Environmental stresses particularly those induced by high soil salinity, heat, heavy metals, drought and freezing severely reduce growth of plants (Ngugi *et al*., 2022). Drought stress happens when the water availability in the soil is less than the requirements of the plant (Hoseini *et al*., 2023) and reduces leaf area index, growth of roots, nutrients uptake, rate of respiration and photosynthesis (Yuan et *al*., 2016; Wenter *et al*., 2018; Tombesi *et al*., 2018; Zahra *et al*., 2023).

Drought stress brings about changes in anatomy and causes accumulation of secondary metabolites in plants (Jogawat *et al*., 2021). Higher phenols production in roots and leaves of plants under the conditions of low soil moisture stress has been studied in plants (Hura *et al*., 2015). Phenolic compounds play diverse roles in development and growth adding to the tolerance of plants under the conditions of low water availability (Pratyusha, 2022). Reactive oxygen species (ROS) formed during drought stress are stabilized by phenolic compounds (Akram *et al*., 2017). Moreover, the *de novo* synthesis of antioxidant enzymes like superoxide dismutase (SOD) and peroxidase (POD) also takes place under drought stress to protect biomolecules of the cell from destruction by ROS (Kusvuran *et al*., 2019).

Salicylic acid is an important phenolic compound and has been found with various physiological actions in plants under drought stress conditions. The role of salicylic acid in the translocation of solutes, ionic uptake, photosynthesis, gene expression, flowering and stress tolerance of plants has been well explored (Latif *et al*., 2016; Wang *et al*., 2019; Abbass *et al*., 2019). Salicylic acid beneficially effects physiology and morphology of crop plants in conditions of low soil moisture availability (El Sherbiny *et al*., 2022; Kour et al., 2022) by maintaining the concentration of leaf proteins and other osmolytes (Khan *et al*., 2012). Salicylic acid protected photosynthetic pigments of pea plants under drought conditions and resulted in higher seed yield (Anwar *et al*., 2025).

Coriander (*Coriandrum sativum* L.) belonging to family Apiaceae is an annual herb. Its vegetative parts and fruit contain essential oil with medicinal value. Leaves of Coriander are rich in vitamin C, vitamin K, and vitamin A. It is used in digestive problems and stomach disturbances (Carruba *et al*., 2006). According to the earlier researcher demonstrated that drought stress significantly decreased the production of fresh matter and seed yield of coriander (Silva *et al*., 2013).

The present investigation was to explore the possible role of salicylic acid on the endogenous phenolic compounds regulation and antioxidant enzymes defense system of coriander and their potential relationship with drought stress tolerance of *C. sativum*.

MATERIALS AND METHODS

The seeds of coriander variety Dilpazir were thoroughly washed with a solution of mercuric chloride (0.2%w/v) for five minutes followed by three times washing with dH_2O. The seeds were sown in plastic container (10x12 cm^2) filled with a mixture of sand, clay and farmyard manure (3:2:1). The experiment was performed in a glass house during the coriander growing season at the department of Plant Biodiversity University of Science and Technology Bannu. When the plants reached to four leaves stage than they were exposed to constant drought stress by disconnecting the normal supply of water for 7 days. Plants in control received normal irrigation when required.

A stock solution of salicylic acid was made by dissolving the prerequisite quantity of SA in 1 ml ethanol. The volume of ethanolic solution of SA was increased to 0.1 L with distilled water. The stock solution of SA was diluted to obtain final concentrations of 0.5 and 1 mg/L. Salicylic acid was applied to leaves as a pray one day before the beginning of drought stress period. Each treatment had three replicates. The treatments were: Control- supplied with normal irrigation when required, Drought stress, Foliar applied salicylic acid at 1mg/L, Foliar applied salicylic acid at 0.5 mg/L, Foliar applied salicylic acid (1 mg/L) + Drought stress, Foliar applied salicylic acid (0.5 mg/L) + Drought stress.

After the completion of drought stress period for 7 days moisture content in soil was determined on the dry weight basis of the soil.

$$Soil\ moisture\ content\ (\%) = \frac{Soil\ fresh\ weight(g) - soil\ dry\ weight\ (g)}{soil\ dry\ weight\ (g)}$$

During the Drought period the soil moisture level decrease up to 7%.

After drought stress plants in all the treatments were uprooted and examined for the following growth parameters.

Seedlings weight both fresh and dry weights were measured with a balance (TX-323 L Shimadzu Japan). Gao (2000) method was used for leaf relative water content (LRWC) determination. After determination of fresh weight, leaves were kept in distilled water for overnight at room temperature and their turgid weight was measured. Later on the leaves were dried to constant weight at 72 ^oC for three days and their dry weight was measured.

$$LRWC\ (\%) = \frac{Fresh\ weigh\ of\ leaf\ (g) - Dry\ weight\ leaf\ (g)}{Turgid\ weight\ of\ leaf\ (g) - Dry\ weight\ of\ leaf\ (g)} \times 100$$

Green pigments of leaves like chlorophyll *a* and *b* contents were assessed according to the method of Arnon (1949). Lowery (1951) procedure was adopted for the estimation of total soluble proteins content of leaves using BSA (Bovine Serum Albumen) as a standard.

This study used protocol of Swain and Hillis (1959) for the determination of total soluble phenolics content. The 100 mg fresh pieces of leaves or roots were treated with methanol for overnight. The solutions were then centrifuged 8000 rpm for 15 minutes and supernatant was collected in a fresh test tube. The 50 µL of the supernatant was diluted to 250 µL using aqueous methanol (50%) and further added with 250 µL folin-ciocalteau reagent. The solution was kept for 3 minutes without shaking. The solution was later mixed 500 µL sodium bicarbonate solution (7.5%) and kept in dark for one hour at 25^{o}C. The absorbance of the reaction mixture was noted at 765 nm using a UV-visible spectrophotometer (SP-300 Japan). The samples absorbance values were compared with absorbance values of gallic acid solutions of different concentrations (1 to 32 µg/mL). The unit defined for the content of total soluble phenolics content was µg gallic acid equivalents/g fresh weight.

To extract the antioxidant enzymes, a 500 mg frozen leaves of coriander were homogenized in a 3ml cold solution of potassium phosphate buffer (50 Mm) having EDTA (1Mm), dithiothreitol (1 Mm) and polyvinyl pyrrolidone (2%). After centrifuging the homogenate for 15 minutes at 13000 g, the supernatant was used for the POD and SOD tests. The activity of SOD was calculated by the method of Beyer and Fridowich (1987). A total of 20 µl of the supernatant containing enzyme was shaken with 50 mM pH 7.8, 9.9 mM L-methionine, 57 µM nitro blue tetrazolium, and 0.025% Triton-X-100 potassium phosphate buffer. To start the reaction, riboflavin solution (10 µL) was added to the mixture and placed under a fluorescent light for 10 min. The control samples were placed in the complete dark. The samples absorbance was determined at 560 nm (Spectrophotometer, SP-3,Japan). An activity of SOD was stated as Units mg^{-1} protein.

The peroxidase activity was calculated by the method of Ranieri *et al*.(2000) which is based on the spectrophotometric measurement of oxidation of o-dianisidine (3, 3-dimethoxy benzidine) at 460 nm. The reaction mixture was composed of phosphate buffer (20 mM, pH 5.0), dianisidine (1 mM), H_2O_2 (3 mM) and 100 µL of enzyme extract. The activity of POD was expressed in units (µmoldianisidine oxidized per minute).

For essential oil extraction whole plants of coriander (5g) from each treatment were ground in a blender separately. Essential oil was extracted by water distillation method (Hassan and Ali, 2014).

Essential oil (%) = volume of oil / weight of sample x 100 ----------------------------------Eq. 2

Statically analysis

The data significance was determined using one way analysis of variance (Steel and Torrie, 1984) and all the calculated means were compared using least significant difference test. The above tests were performed using STATISTIX-9. The standard error mean values (n= 3) were calculated using MS excel (MS office version 13).

RESULTS AND DISCUSSION

In comparison to control drought stress drastically decreased fresh weight and dry weight of coriander seedling (p=0.00) (Table 1). In a non-stress environment SA (0.5 mg/L) application boosted seedling fresh weight over control. The foliar use of SA at both the concentrations lessened the negative impact of drought on seedling weight. These results agree to the former findings of Sure *et al*. (2011) that drought-induced inhibition in shoot weight of *Cucurbita pepo* L. was ameliorated by foliar application of SA. Water stress is related with the lessening in metabolic activity of plants which ultimately leads to lower growth rates (El Tayeb and Ahmad, 2010). The SA regulate water budget of plants and therefore protect plants from the adverse effect of drought stress (Ullah *et al*., 2012). It is also worthy to note that low soil moisture stress results in the arrest of cell division of cambial cells, leading to lower biomass production (Giordano *et al*., 2021).

Table 1. Impact of salicylic acid on morphological and physiological growth attributes of Coriander under drought stress.

Treatment	Seedling Fresh Weight (g)	Seedling Dry Weight (g)	Relative Water Content (%)	Chlorophyll *a* (mg/g F.W)	Chlorophyll *b* (mg/g F.W)
Control	0.243±0.013[b]	0.024±0.004[ab]	88.00±3.511 [a]	0.047±0.002 [d]	0.016±0.003 [b]
Drought	0.118±0.046 [c]	0.018±0.002 [bc]	63.33±1.763 [b]	0.027±0.001 [e]	0.019±0.002 [b]
Salicylic acid (1 mg/L)	0.275±0.024[b]	0.022±0.003[ab]	84.67±4.666 [a]	0.085±0.001[bc]	0.039±0.005 [a]
Salicylic acid (0.5 mg/L**)**	0.370±0.038 [a]	0.024±0.006 [a]	84.67±1.456 [a]	0.113±0.004 [a]	0.045±0.005 [a]
Drought+ Salicylic acid (1 mg/L)	0.223±0.028 [b]	0.015±0.001 [c]	84.66±1.522 [a]	0.097±0.006 [b]	0.043±0.002 [a]
Drought+ Salicylic acid (0.5 mg/L**)**	0.234±0.008 [b]	0.025±0.003 [a]	85.00±3.844 [a]	0.081±0.009 [c]	0.049±0.008 [a]
LSD	0.0587	6.127	9.4508	0.0144	0.0132

When compared with plants in control drought stress negatively influenced LRWC ($p<0.05$). The LRWC was retained by foliar spray of both 1 and 0.5 mg/L SA (Table 1). Leaf relative water content is a reliable indicator for determining water status of plants (Ober *et al*., 2005). The reduction in LRWC by imposed drought stress is common among plant species (Valentovic *et al*., 2006). Foliar spray of SA effectively retained the LRWC of maize plants under low availability of soil moisture (Latif *et al*., 2016).

Chlorophyll *a* content in coriander leaves ($p<0.05$) was decreased by imposed drought stress. However, it has no influence on the content of chlorophyll *b*. Under control conditions, both the concentrations of SA significantly augmented the content of chlorophyll *a* and chlorophyll *b* over control. The SA at both the concentrations was significantly effective and protected chlorophyll *a* from inhibitory effects of drought stress. Previous studies have documented that low soil moisture stress inhibits the production of green pigments in plants (Ullah *et al*., 2012). The

enzymes activity like Rubisco and PEP carboxylase is reduced due water stress. (Song *et al*., 2023). The application of SA increase the chlorophyll content were reported by other research workers such as Alam *et al*., (2022) in *Trtiticum aestivum* and Hayat *et al*. (2008) in tomato. The SA application increases the chlorophyll content and increased plant productivity and yields due higher chlorophyll content (Roa *et al*., 2012). The present results confirmed that the application of foliar spray of SA inhibits of drought stress on chlorophyll content of coriander.
Drought stress stimulated soluble proteins production in coriander leaves. Both in non-stress and imposed drought stress the outcome of foliar spray of SA on soluble proteins content was stimulatory (**Table 2**). The accumulation of proteins assists in osmotic regulation which makes the plants tolerant to drought stress (Ma and Turner, 2006). The stimulatory effect of SA on soluble proteins content in conditions of drought has been reported in plant species like wheat (El Tayeb and Ahmad (2010) and Canola (Ullah *et al*., 2012).

Table 2. Impact of salicylic acid on biochemical growth Parameter of Coriander under drought stress.

Treatment	Super oxide dismutase (units/g FW)	Peroxidase (O.D/min/g FW)	Total leaf soluble Protein (mg/g F.W)	Shoot Phenolics (µg gallic acid eq./g f.w)	Root Phenolics (µg Gallic acid eq./g) f.w)
Control	0.08±0.011[d]	0.433±0.066[c]	65.33±3.179[e]	75.25±1.826[d]	11.847±0.244[e]
Drought	0.2±0.202[b]	1.33±0.057[b]	117.67±3.282[c]	87.70±6.016[cd]	19.637±0.592[d]
Salicylic acid (1 mg/L)	0.153±0.017[bc]	0.6± .066[c]	84.33±1.763[d]	98.68±5.011[c]	25.853±1.26[c]
Salicylic acid (0.5 mg/L)	0.121±0.03[cd]	0.466±0.145[c]	69.66±2.081[e]	89.073±6.38[cd]	21.037±0.456[d]
Drought+ Salicylic acid (1 mg/L)	0.516±0.034[a]	2.366±0.133[a]	171±1.154[a]	146.34±3.955[a]	31.037±0.7[a]
Drought+ Salicylic acid (0.5 mg/L)	0.453±0.020[a]	2.233±0.240[a]	157±1.452[b]	127.39±1.25[b]	28.197±0.57[b]
LSD	0.4151	0.0709	7.0912	13.927	2.1930

Similar letters are non significant in each column according to Duncan Multiple Range Test at $p < 0.005$.

Total soluble phenolics content in leaf was not influenced by drought stress. However, both the concentrations of SA enhanced the production of phenolics in leaves of drought subjected plants. The SA at 10^{-4}mol/L was more effective in the increase of phenolic compounds in leaf **(Table 2).**

Unlike leaves roots of coriander showed an increase in total soluble phenolics content upon exposure to drought stress. In non-stressed conditions, foliar application of SA increased total soluble phenolics content. However, the SA beneficial effect on roots phenolic was more noticeable in drought treated plants. The supportive role of SA on the production of phenols in root was higher at 1 mg/L **(Table 2)**.

The accumulation of phenolic compound in response to drought stress has been confirmed in *Prunella vulgaris* (Chen *et al*., 2011) and winter triticale (Hura *et al*., 2015). Hale *et al*. (2005) stated that during drought stress the phenolic compounds build up at the expense of carbon fixed during photosynthesis, lowering the supply of carbohydrates and hence slowing down plant growth. The higher accumulation of phenolic compounds in roots might be because that root is the first organ of a plant which is directly in contact with soil moisture. The SA stimulatory effect on endogenous phenolics content could be correlated with the phenolic nature of SA. The increased accumulation of phenolic compounds by SA under drought stress may be a mechanism involved behind its beneficial effect under drought stress (Latif *et al*., 2016).

In comparison to plants in control drought treated plants exhibited significantly higher antioxidants enzymes activity in leaves ($p<0.05$). Foliar application of SA under drought stress resulted in higher SOD and POD activity **(Table 2)**. Greater SOD and POD activity under drought stress condition was reported in leaves of *Thymus daenensis* **(**Bahari *et al*., 2015). The activity of SOD neutralizes the O^{-2} free radicles to converts into H_2O_2 and H_2O_2 changes into H_2O as a result of POD role (Irawan *et al*., 2023). Hayat et al. (2005) stated that by the application of SA the increase of SOD and POD activity was found in *Triticum aestivum* during the drought stress.

Impact of salicylic acid on essential oil content of Coriander under drought stress

Essential oil content in leaves of coriander was decreased by imposed drought stress (Fig. 1). The non-stressed plants treated with SA at 1 mg/L and 0.5 mg/L had higher essential oil content than those in control group. More importantly SA also maintained normal essential oil content in leaves of plants exposed to drought stress. Low soil moisture effects essential oil yield of aromatic plants (Mohammad *et al*., 2002; Ekrena *et al*., 2012). This reduction in essential oil content may be because that water deficiency stress inhibits normal metabolism of plants leading to biosynthesis of essential oils (Hassan and Ali, 2014). Abiotic factors like changing climate significantly influenced essential oil content of *Vitex trifolia* var. *Purpurea* (Dehsheikh *et al*., 2019). The regulation of metabolism and biosynthesis of osmolytes, antioxidants and other secondary metabolites by the application of SA during water deficit stress is reported (Jaleel et al,. 2009). We confirmed that SA improved essential oil content along with phenolics content of coriander plants exposed to drought stress.

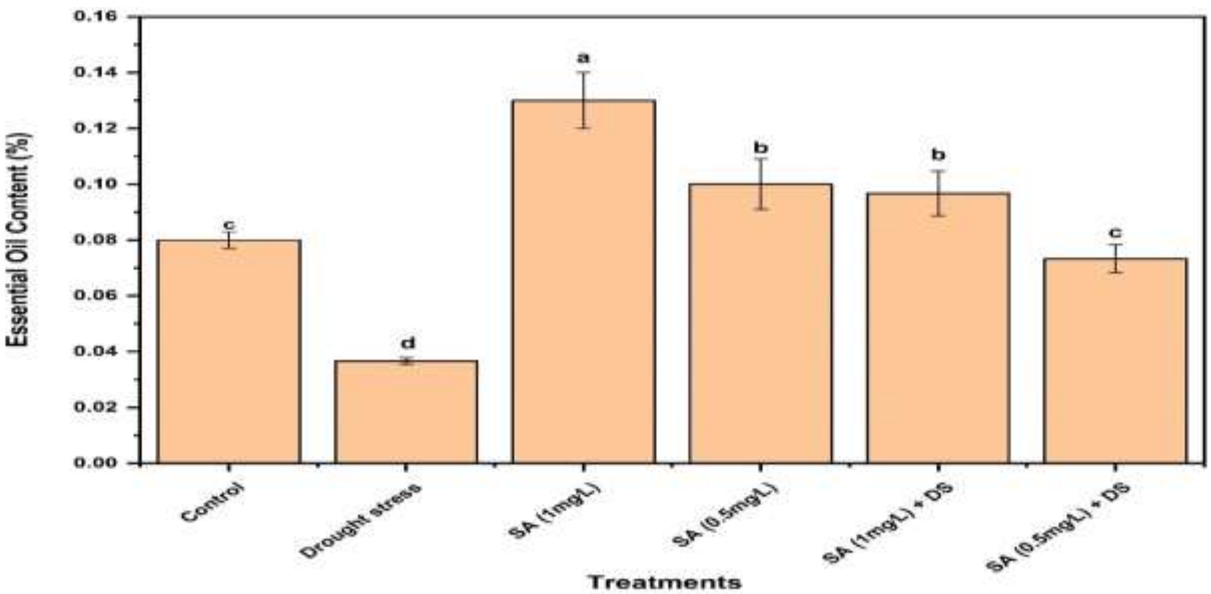

Fig. 1. Impact of salicylic acid on essential oil content of Coriander under drought stress.

Conclusion

Foliar application of SA improved tolerance of coriander to drought stress. Foliar spray of SA improved production of phenolics and increased activity of antioxidant enzymes in coriander leaf. The higher phenolics content and antioxidant enzymes activity might contribute to the drought stress tolerance of this plant species. This study provided information about the sustainable and eco-friendly increase in essential oil content of coriander further adding to its medicinal value.

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