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# Impact of Exogenous Applications of Salicylic Acid on the Tolerance to Drought Stress in Pepper (Capsicum Annuum L.) Plants

**zahra khazaei** (zahrakhazaei55@yahoo.com)
University of Mohaghegh Ardabili https://orcid.org/0000-0001-7528-1570
**Asghar Estaji**
University of Mohaghegh Ardabili

**Original article**

**Keywords:** Antioxidant properties, Electrical conductivity, Total phenolic content

**Posted Date:** December 1st, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-116192/v1

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## Abstract

**Background:** Drought is also one of the most widespread abiotic stresses that adversely effects the growth and development of plants. To investigate the effect of salicylic acid and drought stress on several physiological and chemical reactions in sweet pepper plants, the experiment was achieved as a factorial based on a completely randomized design in greenhouse. Drought stress levels were non-stress conditions (irrigation with field capacity), moderate stress (30% field capacity irrigation) and intense water stress (60% field capacity irrigation) and three concentrations of salicylic acid included 0 (as control), 0.5 and 1 mM were sprayed on the plant in three to four leaf stages.

**Results:** The results showed that drought decreased fresh and dry weight of shoots and roots, leaf relative water content (RWC), fruit diameter and length, the index including chlorophyll and leaf area and increased electrical conductivity (EC), antioxidant activity, total phenolic content, ascorbate, polyphenol oxidase (PPO) and ascorbate peroxidase (APX) activity. After application of foliar salicylic acid, all of the above parameters, except the electrical conductivity content, increased.

**Conclusions:** From the results of this experiment it is concluded that salicylic acid provides a better tolerance for drought stress in pepper plant through its influence on vegetative, biochemical and physiological characteristics.


## Background

Water stress is a limiting environmental parameter that restricts crop production, especially endangered plant species, and can negatively impact on processes such as photosynthesis, plant growth and yield (Shen *et al.* 2014). The drought decreases relative water content (RWC), transpiration rate and leaf water potential in plants. One of the most important effects of drought is the generation of oxidative stress that causes an imbalance between the production of reactive oxygen species and enzymatic and non-enzymatic antioxidant defense systems, (Farooq *et al.* 2009) and injures photosynthetic apparatus and disrupts electron transport in chloroplasts and other cell compartments, it can induce generation of hydroxyl radicals ($^{\bullet}OH$), superoxide anion radicals ($O^{2-}$), singlet oxygen ($^1O_2$) and hydrogen peroxide ($H_2O_2$) (Hayat *et al.* 2008; Demiralay *et al.* 2013). ROS react with cellular components such as proteins and lipids of the cell membrane and other; it results in oxidative damage in the plant (Farooq *et al.* 2009; Demiralay *et al.* 2013). The plants have enzymatic antioxidant systems such as ascorbate peroxidase (APX) (Kadioglu *et al.* 2011) and polyphenol oxidase (Qados 2015). A study showed that the cell membranes are prime and sensitive sites in cells (Korkmaz *et al.* 2007). The drought stress causes membrane disruption and leads to increasing electrolyte leakage (Still and Pill 2004). The plant's antioxidant capacity under stress conditions is insufficient to reduce the harmful effects of oxidative, so plants produce signaling molecules such as jasmonic acid, ethylene and salicylic acid (Kadioglu *et al.* 2011). Plant Growth Regulators (PGRs) are inexpensive and easy-to-use compounds, they can induce to enhance water use efficiency and adapt to drought stress in the plant.

Salicylic acid (SA) is considerable PGRs and known as a signaling molecule in plants that induces the immune system in plants (Khan *et al.* 2015). External application of SA in processes such as seed germination (Kalai *et al.* 2016), ion uptake and transport (Shi and Zou 2008), photosynthesis (Liu *et al.* 2014) and enzymatic properties (Guo *et al.* 2007; Ahmad *et al.* 2011) plays a role in the plant. Hossein et al. (2007) reported that SA contributed to an increase in growth rate and photosynthesis in maize plant. In another study (Habibi 2012) reported that SA enhanced photosynthetic activity and stomatal conductance in barley under stress conditions. In one study Kadoglu et al. (2011) stated that salicylic acid modulates plant responses and leads to more resistance to drought stress. Salicylic acid plays an key acting in cell membrane balancing (Yang *et al.* 2004) by reducing electrolyte leakage from plant membranes as well as enhancing growth processes in salicylic acid-treated plants compared to control plants (Hayat and Ahmad 2007) Also, Salicylic acid increases antioxidant enzymes activity such as APX and PPO and reduce the harmful effects of reactive oxygen species caused by oxidative stress (Korkmaz *et al.* 2007; Qados, 2015).

Sweet pepper (*Capsicum annuum* L.) is a rich source of essential vitamins and minerals. On the other hand, pepper fruit contains high levels of antioxidants and beneficial substances such as vitamin C, carotenoids and phenolic compounds. It also contains high concentrations of potassium (Bosland and Votova 2000). These compounds has nutritional and antioxidant capacity (Kavikshore *et al.* 2005). Consumption of sweet pepper similar to tomato has an effective role in the prevention of heart disease because of high antioxidant and lycopene content (Shilpi and Narendra 2005). According to the skin color, sweet pepper fruit contains different amounts of vitamin C (Bosland and Votova 2000). Fresh pepper fruit has been introduced as one of the high vitamin C herbs in the vegetable chain (Shewfelet and Bruckner 2000).

Our aim was to investigate the effect of external application of salicylic acid on reducing drought-induced oxidative damage in sweet pepper. Investigating and comparing seedling responses to salicylic acid has shown that it can reduce the harmful impacts of reactive oxygen species by enhancing the antioxidant defense system and ultimately increase drought resistance in pepper plants (Caverzan *et al.* 2019).

## Materials And Methods At The

2.1. Experimental details

The pepper plants (*Capsicum annuum* L.) were used as the experimental material. This work was carried out in a completely randomized design (RCD) with factorial arrangement and was replicated thrice. The main factors were different levels of SA concentrations and drought. This research was carried out an experimental greenhouse at the Faculty of Agriculture, University of Ilam, Iran. The experiment was conducted during four months. The sweet pepper seeds were obtained from the Faculty of Agriculture. Before sowing, seeds sterilized by immersing in 1% sodium hypochlorite for 10 min before being washed with tap water for 1 min. Then, the seeds were sown in plastic pots (23×20 cm). The pots were filled the same amount of sand, garden soil and leaf mold (1: 1: 1) mixture. After that, each pot weighed 7 kg. The soil samples were analyzed to determine different soil properties (Table 1).

The relative humidity (R.H. %) and mean temperature during the growth seasons were 60–70% and 18/ 25 °C (day/ night) respectively. SA was used at three levels, namely 0 (sprayed with distilled water as acontrol), 0.5 and 1mM. The SA was sprayed upper and lower leaves of fourth leaf stage. Tween-20 was used as a surfactant. The first and second spray application of SA was at three days before and two weeks after the drought stress began. In the early stages of plant growth, all pots were watered until field capacity. Accordingly, it lasted approximately four months three days after the foliar spray until sampling. The experiment consisted of three levels of drought stress: full irrigation (control group), 60% and 30% of field capacity, moderate and severe stress respectively. We have not used any fertigation in this experiment. All pots were weighed on a daily basis. These conditions were contended until the end of the investigation.

For the determination of physic-chemical parameters, 27 plants were harvested in the green stage (80% maturity). Leaf samples were harvested by three replicates and each replicate was obtained from three pots, and therefore nine plants existed in each treatment group. The samples were quickly frozen in liquid nitrogen. They were stored at -80 °C.

**Growth Parameters**

The leaf area (LA) of leaves was measured using an area meter (AM 300 Bioscientific Ltd.UK) fully grown leaves that expanded from the main stem (Phimchan *et al.* 2012). The leaf chlorophyll index (CHLI) was assessed according to the method described in the study of Wang *et al.* (2017) from the last fully expanded leaf of sweet pepper by SPAD502. The shoots fresh weight of pepper plants were assessed by a digital analytical balance. The dry weight of shoots were determined after being dried in an oven at $60^{\circ C}$ (Ahmed *et al.* 2014). The roots of the sweet pepper plants were carefully separated and then washed thoroughly with distilled water several times and then measured with a digital analytical balance. Then, they were placed in an oven at $60^{\circ C}$ to assess the dry weight of roots (Ahmed *et al.* 2014). A method by Barranco *et al.* (2005) was applied for determination of leaf relative water content (RWC). According that, the fresh leaves were cut into 0.1 g disks. The contaminants and residues of samples were removed thoroughly with distilled water. Then, the leaf samples were placed to the test tubes with 10 ml distilled water. The tubes were placed for 30 min at $30^{\circ C}$, subsequently the initial electric conductivity of the solution ($Ec_1$) was measured. For calculating secondary electric conductivity ($Ec_2$), the tubes heated for 15 min at 100°C in bath of water. finely; the total electrical conductivity was calculated using the formula:

EL (%) = $(EC_1/EC_2) \times 100$.

**Fruit physical properties**

The morphological characteristics of pepper fruits such as length and diameter of fruit were measured. The measurements of diameter and length of the fruits were taken in maximum width and length. All measurements were by using a Caliber (Thuy and Kenji 2015).

**Determine relative electrical conductivity**

This test was performed by referring to the method developed by Deshmukh *et al.* (1991). The fresh leaves Cut (0.1 g) by disk and remove contaminants with distilled water. Then the leaf samples were placed to the test tubes containing 10 ml of distilled water and incubated for 30 min at $30^{\circ C}$. Then the initial electric conductivity of the solution ($Ec_1$) was measured. Subsequently the tubes heated for 15 min at $100^{\circ C}$ in a bath of water and the secondary electric conductivity ($Ec_2$) was calculated. Eventually the total electrical conductivity was determined by the following formula:

EL (%) = $(EC_1/EC_2) \times 100$

**Measurement of Non-enzymatic antioxidants**

**Measurement of AsA content**

A modified procedure by Luwe *et al.* (1993) was estimated for determination AsA content of pepper leaves. Initially, the samples pepper leaves (0.5 g) were poured by mortar and pestle in liquid nitrogen ant then homogenized in cold trichloroacetic acid (TCA, 1% w/v) and centrifuged at 12000×g for 20 min at $4^{\circ C}$. The supernatant was mixed with 50 µL potassium phosphate buffer mixture (0.95 ml, 100 mm, pH 7.0) along with ascorbate oxidase (1 µl of 1 $\mu l^{-1}$ unit). Finely, the absorbance was read at 265 nm.

**Assessment of Total phenolic content**

The total phenol content was estimated per the method of Kahkonen *et al.* (1999). The leaves of fresh pepper (0.4 g) were homogenized in 4 ml of methanol and 0.1 Mol $l^{-1}$ HCl mixtures. The mixture was centrifuged at 15000×g for 20 min at $4^{\circ C}$. The supernatant was mixed with distilled water (1:10) and by adding 2 ml diluted solution with 400 µl supernatant. The mixture was then added 1.6 ml of sodium carbonate ($Na_2CO_3$ 7.5%) to the above solution. The solution was stored in the dark for 30 min and centrifuged at 5000×g for 5 min. Eventually, the absorbance of the mixture was measured at 765 nm. The total phenolic content was estimated by Gallic acid mg $ml^{-1}$ fresh material.

**Antioxidant property**

The DPPH was used to determine antioxidant activity of pepper leaves as described by Ab *et al.* (1998). The fresh leaves (0.2 g) were ground homogeneously with a mortar and pestle with 2 ml of ethanol at $4^{\circ C}$. About half of the solution was blended with a solution consist of 0.5 ml of 100 mm acetate buffer (pH 5.5) and 0.25 ml of 0.5 mM DPPH. The absorbance was recorded at 517 nm after 30 min. The antioxidant properties were calculated using the formula:

$$\text{Antioxidant capacity (\%)} = \left(1 - \frac{ASample\ (517nm)}{AControl\ (517\ nm)}\right) \times 100$$

#### Assaying the activity of antioxidant enzymes

#### Preparation of extract:

The pepper plant leaf was crushed in a mortar containing 50 ml of sodium phosphate buffer and then, centrifuged at 16,000 g at 10°$^{C}$. The supernatant was to determine antioxidant enzyme activities APX and PPO.

#### Measurement of polyphenol oxidase activity

Reaction mixture containing 900 µl of catechol and 40 µl of 0.01 M sodium phosphate buffer with pH 6.8 was prepared and then 100 µl of enzyme extract was added. Then, the absorbance was recorded at 400 nm in 25$^{°C}$ for 3 min (Jiang *et al*. 2002**)**.

#### Assaying of ascorbate peroxidase enzyme

For this assay, 0.1 ml of enzyme extract was added to a mixture containing 0.1 ml of 0.5 mM ascorbate, 0.2 ml of $H_2O_2$ 1%, 2.5 ml of 50 mM potassium phosphate buffer and 0.1 mM EDTA and then the absorption was recorded at 290 nm at 25$^{°C}$ (Asada 1992).

#### Statistical analysis

All statistical analyses were performed by analysis of variance (ANOVA) using SPSS statistical 10 software. Differences between treatments were separated by Duncan's multiple range tests. The analyses were carried out to determine significant variation between the means at a significance level of $P < 0.05$.

## Results

#### Correlation coefficient

The correlation coefficient was used for determining the natural and extensive relationships between the studied traits in this research. According to the results, some of the studied traits were significant (Table 2). A positive significant correlation was reported between fresh weight of shoot with dry weight of shoot, fresh weight of root, dry weight of root, RWC, fruit diameter, fruit length and leaf area index. Also, fresh weight of shoot had a negative significant correlation with chlorophyll index. Meanwhile, the dry weight of shoots had a positive significant correlation with the fresh weight of roots, dry weight of roots, RWC, fruit diameter, fruit length, but there was a significant negative with chlorophyll index. In present study, fresh weight of roots correlated positively with dry weight of roots, relative water content, fruit diameter and fruit length. Meanwhile, a high significant correlation was found between root dry weight with relative water content, fruit diameter, fruit length, but had a negative significant correlation with leaf area index. In addition, we reported that a significant positive correlation was found between RWC with fruit diameter, fruit length, leaf area index, but had correlated negatively with chlorophyll index. Also, positive and significant correlation occurred for chlorophyll index with EC and ascorbic acid, but had significant negative correlation with fruit diameter and fruit length. A positive and significant correlation was observed for fruit diameter with fruit length and leaf area index, but a negative significant correlation was observed for ascorbic acid. A positive and significant correlation was observed for fruit length with leaf area index and negative significant correlation was observed with polyphenol oxidase, ascorbic acid and EC. Meantime, we reported that a significant positive correlation was observed between polyphenol oxidase with ascorbate peroxidase, ascorbic acid, EC, total phenolic content and antioxidant capacity. Ascorbate peroxidase shows a significant positive correlation with ascorbic acid, EC, total phenol and antioxidant activity. Also, positive and significant correlation occurred for Ascorbic acid with EC, total phenol and antioxidant activity. We reported that a significant positive correlation was observed between EC with total phenol and antioxidant activity. Ultimately, correlation analysis showed that total phenol has a positive and significant correlation with AsA. In a study on apple, Pearson correlation analysis in apples exhibited that the measured antioxidant activity can be attributed to the total phenolic measurement duo to relationship between each antioxidant and total phenolic content was very significant (Candrawinata *et al.* 2014).

#### Growth parameters

According to the data analysis of the present study (Table 3 and 4) drought stress and salicylic acid had a significant effect on fresh and dry weight of the shoots and roots, fruit length and diameter, leaf area index, chlorophyll index. Statistical results showed that with increasing drought stress, fresh and dry weight of the shoots and roots, fruit length and diameter, leaf area index, chlorophyll index decreased. As shown in Tables 3 and 4, foliar application of SA markedly increased the parameters mentioned above at all drought levels. Furthermore, the interaction effects of drought and salicylic acid treatments on these traits were significant except dry weight of shoot, fresh weight of root, fruit diameter, length fruit, chlorophyll index, leaf area index (Fig 1a and 1b and table 4).

#### Relative water contents (RWC)

In the present study, RWC content in pepper plants under drought stress was significantly decreased. As the stress increased, this trait decreased (Table 3). Foliar application of SA significantly decreased the unfavorable effects of drought stress and thereby increased this trait (Table 3). In this feature, there was a significant interaction between drought stress and salicylic acid (Fig.1d).

#### Electrical conductivity

Results showed that drought stress and SA had a significant effect on electrolyte leakage. Drought stress increased electrolyte leakage into the intercellular space.

The highest enhance was observed at the severe drought stress as compared with controls. The foliar application of SA markedly reduced the electrical conductivity in stressed plants (Table 4). Also, the interactive effect of salicylic acid and drought stress was significant so that with increasing drought stress, electric conductivity content increased and after the use of SA has decreased (Fig. 1c).

#### Ascorbic acid

Different concentrations of salicylic acid significantly increased the amount of ascorbic acid in pepper plants under drought and control conditions (Table 5) and this increase was greater in plants treated with salicylic acid under drought stress than non-stress conditions. Interaction of drought and salicylic acid treatments had significant effect on ascorbic acid content and showed that ascorbic acid content in pepper plants increased with enhancing salicylic and acid drought stress (Fig. 1e).

#### Total phenolics

According to the data analysis, drought stress had a significant effect on the total phenolic content of pepper plants (Table 5). After applying different concentrations of salicylic acid, total phenolic content in pepper plants was significantly under stress and control conditions (Table 5). The interaction between drought stress and salicylic acid on total phenolic content was significant and showed that total phenol content increased with increasing drought stress and salicylic acid concentration in pepper plants (Fig. 2c).

#### Antioxidant capacity

The endogenous content of antioxidant activity remarkable increased in pepper plants with enhancing levels of drought compared to control conditions (Table 5). It markedly enhanced content of antioxidant activity in pepper plants when foliar SA was applied in pepper plants (Table 5). The interactive impact of drought stress and salicylic acid on antioxidant activity was significant and showed that antioxidant activity in pepper plants increased with increasing drought stress and salicylic acid (Fig 2d).

#### Antioxidant Enzyme Activities

To inhibit reactive oxygen species in pepper plants under drought stress, their antioxidant system was activated and enzymes such as PPO and APX were significantly increased under both drought and SA treatments (Table 5). As shown in Table 5, SA treatment stimulated both antioxidant enzymes more than control plants and the activity of these enzymes increased with increasing SA concentration. The interaction between drought stress and salicylic acid on antioxidant enzymes was significant as the amount of these enzymes increases with increasing drought and salicylic acid (Fig. 2b and a).

## Discussion

Drought is one of the main agents restricting the growth rate and physiological processes of plants (Loutfy *et al.* 2012). Agents such as plant species and genotypes, period of time and severity of drought stress, its age and developmental stage play a role in drought stress response. Drought stress can be evaluated by its effects on fruit morphological properties (El-Mageed *et al.* 2016). The growth of plant is caused by cell division, growth and differentiation. Drought stress reduces cellular turgor and disrupts cell growth processes and ultimately leads to poor plant growth. Plant growth regulators can be used to maintain proper water balance in the plant under drought conditions (Fahad *et al.* 2017).

In this study, results showed that drought stress decrease growth parameters such as fruit length and diameter of pepper plants, while SA increases growth parameters under drought stress. A study by Fariduddin *et al*. (2003) showed that the external spray of Salicylic acid increased growth rate in *Brassica juncea* plants and another study showed that salicylic acid was involved in processes such as enhancement of nutrient uptake (Yildirim *et al.* 2008), induction of root formation (Khan *et al.* 2003; Shen *et al.* 2014) and increased cell division in the apical region of the root meristem and eventually, it results increment of plant growth Sakhabutdinova *et al*. (2003) which is consistent with our findings on pepper.

In the present study, drought stress generally reduced LAI significantly (Table 4). This decrease in leaf area most likely resulted in lower light interception Yordanov *et al*. (2003) and a consequent decrease in photosynthesis. Although SA increased LAI significantly (Table 4), it could be via lowering developmental rate or delaying plant maturity Tasgin *et al*. (2003) and suggesting that foliar spray of SA at least partially compensated for harmful effects of drought stress. Thus, Senaratna *et al*. (2000) suggested that application of salicylic acid in bean and tomato plants could induce stress tolerance which is consistent with our findings on pepper plants.

In the present study, shoot and root growth of pepper plants decreased significantly under drought stress (Table 3), the growth decrease is a suitable plant response to moisture depletion Efeoglu *et al.* (2009) and severe water scarcity affects cell elongation (Nonami 1998). Therefore, the growth decrease of pepper plants is a common symptom under drought stress. SA modulates the oxidative effects of stress that cause cell death and acts as a growth signal in cell resistance (Shirasu *et al.* 1997). In a recent study, SA foliar spray reduced the adverse effects of stress and aided the natural growth of pepper plants (Table 1). The role of SA in plant growth can be attributed to its effect on increasing cell division Sakhabutdinova *et al.* (2003) and we can say that, increasing the dry weight of the plant due to SA application indicates its overcoming of adverse effects of drought and improvement of plant growth Senaratna *et al*. (2000), which is consistent with our results in this study.

The results of our study showed that chlorophyll content decreased significantly on pepper plants under drought stress (Table 4). One study Panda *et al*. (2004) stated that reducing carbon efficiency and increasing ethanol and lactate production decreased chlorophyll content under drought stress and could also be due to the effects of chlorophyllase, peroxidase and phenolic compounds. As a result, it destroys chlorophyll. The reduction of chlorophyll content on pepper under drought stress is consistent with the results of Sanchez-Blanco *et al.* (2004) spraying with salicylic acid increased chlorophyll content (Table 4). It can be said that the use of salicylic acid decreases the amount of chlorophyll by inhibiting the activity of chlorophyll oxidase enzymes and thus exacerbate photosynthesis. Tang *et al.* (2017) reported that chlorophyll content decreased under drought stress, but salicylic acid spray increased chlorophyll content, which is consistent with recent study observations.

Relative water content is a suitable factor to assessing the physiological status of water in stress plants (Kadoglu *et al.* 2011). In this study, RWC decreased on pepper plants under drought stress condition (Table 4), but SA leaf spray in these plants increased RWC (Table 4). The other studies have shown that, SA can lead to an increase in RWC under drought; these results are consistent with our observations on pepper plants (Ying *et al.* 2013; Alam *et al.* 2013).

Electrolyte leakage indicates damage to the cell membrane (Guo *et al.* 2006). The changes in cell lysis lead to impaired function as well as that indicate effect of environmental stress on plants. The disruption of cell membrane under environmental stress which can be explained by increase permeability and ion leakage from the membrane, that measured by the flow of electricity (Gupta *et al.* 2000). In this study, the amount of electrolyte leakage was increased by drought treatment (Table 4). A study on tomatoes under drought stresses showed that electrolyte leakage is due to impaired membrane integrity (Still et al., 2004). In this study, SA reduced the electrolyte leakage of pepper plants (Table 4). The results obtained in this study are consistent with the findings Korkmaz *et al*. (2007) and Ying *et al.* (2013) that acetylsalicylic acid reduces musculoskeletal electrolyte leakage. A study states that fall off in electrolyte leakage content by means of SA pretreatment can be related to the amelioration of antioxidant defense system in presence of SA under PEG stress (Liu *et al.* 2016; Abbaspour and Ehsanpour 2016).

Drought stress not only slows the growth of plants, but also changes the course of some metabolic processes. During long-term drought stress, the transfer of substances due to the decrease in available water results in changes in the concentration of some metabolites. As a result, the amount of water-soluble substances such as ascorbic acid increases. Ascorbic acid can prevent the plants from being oxidized to environmental stress due to the removal of free radicals from stress and its role in cellular stimulation and expansion and the absorption of substances into the cell that is effective in improving stress tolerance (Gallie 2013; Shan *et al.* 2011). In our study indicated that AsA content significantly increased under drought stress (Table 5) and exogenous SA played significant role in enhancing non-enzymatic components such as AsA (Table 5). Zhou et al. (2009) indicated that the use of SA resulted in increased AsA content under drought stress. Increased AsA content plays an effective role in maintaining APX activity (Caverzan *et al.* 2019).

Phenols content is a suitable indicator for assessing environmental stress tolerance and improving plant metabolism (Sharma *et al.* 2019). Polyphenols can tolerate stress in plants through light or antioxidant protection (Agati and Tattini 2010). The results of this study showed that this metabolite increased due to application of both drought and SA treatments (Tables 5 and 6). Khalil *et al.* (2018) showed that total polyphenol content in Thymus vulgaris L. plants increased compared to control under salicylic acid as well as drought stress and our results are consistent with this finding.

In a recent study, our observations showed that the amount of antioxidant activity increased with increasing SA concentration and drought levels in pepper plants (Table 5). Application of appropriate concentrations of SA reduces the harmful effects of oxidative stress by improving antioxidant capacity as well as the synthesis of protective compounds in plants (Hayat and Ahmed 2007). One study showed that plants exposed to drought stress exhibited greater total antioxidant capacity at higher PEG concentrations. Salicylic acid increased total antioxidant capacity in control and drought tolerant plants (Abbaspour and Ehsanpour 2016), the results are consistent with the results of this study.

Antioxidant enzymes are important components in ROS elimination (Navrot *et al.* 2007). Plants have an antioxidant system to reduce the damage caused by active oxygen (Qados 2015). Drought stress and salicylic acid treatments increased the amount of both enzymes studied in this study (Table 5). Foliar spray of maize plants with salicylic acid increased the activity of APX antioxidant enzyme under cadmium Krantev *et al.* (2008). In a study by Kang et al., 2003b, the increased activity of APX antioxidant enzyme in cold conditions was due to the increase in H2O2 and decreased by the enzyme and increased plant tolerance to stress.

Siddika *et al.* (2015) showed that the activity of PPO enzyme in wheat increased under drought conditions and in another study it was suggested that PPO enzyme is involved in reducing oxidative damage in plant. It can therefore be an index of adaptation to adverse environmental conditions Siddika *et al.* (2015), which is consistent with our results in this study.

## Conclusion

The results of the current study on pepper showed that SA treatment plays an essential role in plant drought tolerance. As such, the application of the leaf SA by inducing an antioxidant system in pepper plants reduces the harmful effects of drought conditions. In addition, SA plays an important role in plant growth as well as in plant defense-protective responses.

## Abbreviations

RWC
relative water content; APX:ascorbate peroxidase; PPO:polyphenol oxidase; PGRs:Plant Growth Regulators; SA:Salicylic acid; RCD:completely randomized design; LA:leaf area; CHLI:The leaf chlorophyll index; EC:electric conductivity.

## Declarations

**Acknowledgements**

we gratefully thank the University of Ilam for laboratory equipment.

**Authors'contributions**

Zahra Khazaei and Asghar Estaji contributed to the conception and the design of the study. Zahra Khazaei performed all experiments and wrote the manuscript. Asghar Estaji supplemented and improved the manuscript as well as statistical analysis. All authors contributed to manuscript revision and read and approved the submitted version.

**Funding**

All experiments were performed in the Horticultural laboratory of University of Ilam, without external financial support.

**Availability of data and materials**

All relevant data is contained within the manuscript. In addition, raw data from processed data will be made available by the authors, without undue reservation, to any qualified researcher on request.

**Consent for publication**

All participants consented the confidential publication of their contributions in this study.

**Competing interests**

The authors declare that they have no competing interests.

**Ethics approval and consent to participate**

Not applicable.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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## Tables

Table 1: Physico-chemical properties of the experimental soil

| Characteristics | Units | Values |
|---|---|---|
| Moisture content | (%) | 32 |
| P | (ppm) | 3.47 |
| K | (ppm) | 33.63 |
| pH | | 7.3 |
| Sand | (%) | 22 |
| Clay | (%) | 11 |
| Silt | (%) | 67 |
| Soil texture | | Silty loam |
| EC | (ds/m) | 0.7 |
| Organic carbon | (%) | 0.42 |
| Total N | (%) | 0.04 |

| Table 2: correlation coefficients (r) among pepper plants traits | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FWSH (g) | DWSH (g) | FWR (g) | DWR (g) | RWC (%) | CHL | FD (cm) | FL (cm) | PPO (unit $mg^{-1}$protein ) | APX (unit $mg^{-1}$protein) | ASA (μmolg$^{-1}$FW) | EC (%) |
| FWSH | 1 | | | | | | | | | | | |
| DWSH | 0.73** | 1 | | | | | | | | | | |
| FWR | 0.76** | 0.78** | 1 | | | | | | | | | |
| DWR | 0.87** | 0.62** | 0.62** | 1 | | | | | | | | |
| RWC | 0.91** | 0.66** | 0.64** | 0.87** | 1 | | | | | | | |
| CHL | -0.45* | -0.43* | -0.26 | -0.33 | -0.42* | 1 | | | | | | |
| FD | 0.83** | 0.68** | 0.54** | 0.83** | 0.82** | -0.52** | 1 | | | | | |
| FL | 0.90** | 0.67** | 0.62** | 0.74** | 0.86** | -0.57** | 0.89** | 1 | | | | |
| PPO | -0.23 | -0.25 | -0.19 | -0.03 | -0.24 | 0.34 | -0.18 | -0.39* | 1 | | | |
| APX | 0.001 | -0.03 | -0.003 | 0.27 | 0.02 | 0.23 | 0.02 | -0.28 | 0.73** | 1 | | |
| ASA | -0.37 | -0.30 | -0.25 | -0.19 | -0.35 | 0.47* | -0.47* | -0.63** | 0.81** | 0.72** | 1 | |
| EC | -0.18 | -0.2 | -0.14 | -0.06 | -0.24 | 0.38* | -0.28 | -0.43* | 0.87** | 0.74** | 0.87** | 1 |
| TPH | 0.12 | 0.005 | 0.06 | 0.28 | 0.10 | 0.24 | 0.01 | -0.17 | 0.72** | 0.89** | 0.77** | 0.84** |
| LAI | 0.45* | 0.36 | 0.35 | -0.41* | 0.53** | -0.05 | 0.47* | 0.52** | -0.33 | -0.14 | -0.32 | -0.40 |
| AC | -0.08 | -0.10 | -0.05 | 0.04 | -0.08 | 0.36 | -0.13 | -0.32 | 0.82** | 0.78** | 0.84** | 0.90** |

FWSH, DWSH, FWR, DWR, RWC, DF, LF , CHLI, EC, AC, TPC, PPO, APX, ASA and LAI were the abbreviations of Fresh weight of shoot, Dry weight of shoot, Fresh weight root, Relative water content, Fruit diameter, Length fruit, Chlorophyll index. Electrical Conductivity, Antioxidant capacity, Total phenol content, Polypher peroxidase, Ascorbic acid, Leaf area index.

Table 3

Mean comparison of salicylic acid and drought stress effects on morphological parameters of pepper plants

| Treatments | FWSH (g) | DWSH (g) | FWR (g) | DWR (g) | FD (mm) | FL (mm) |
|---|---|---|---|---|---|---|
| Ascorbic acid | | | | | | |
| 0mM (control) | 13.92c | 2.90a | 17.12a | 4.12c | 1.37b | 1.68b |
| 0.5 mM | 17.90b | 3.27a | 20.46a | 7.28b | 1.50b | 1.69b |
| 1 mM | 22.59a | 3.72a | 22.86a | 10.60a | 1.92a | 1.94a |
| Drought stress | | | | | | |
| 0 (control) | 29.98a | 4.29a | 27.36a | 11.72a | 9.38a | 7.22a |
| 30% | 14.50b | 3.55a | 19.09b | 6.58b | 2.14b | 2.86b |
| 60% | 9.93c | 2.05b | 13.99b | 3.70c | 1.76c | 1.68c |

The same letters in each column indicate no significant difference at the 5% probability level in the Duncan test. FWSH, SHDW, RFW, RDW, DF and FL were the abbreviations of Fresh weight shoot, Dry weight shoot, Fresh weight root, Dry weight root, Fruit diameter, Fruit length

Table 4

Mean comparison of salicylic acid and drought stress effects on morphological parameters of pepper plants

| Treatments | LAI ($cm^2$) | CHLI | RWC (%) | EC (%) |
|---|---|---|---|---|
| Ascorbic acid | | | | |
| 0mM (control) | 12.26b | 63.98a | 37.94b | 10.97a |
| 0.5 mM | 12.32b | 65.36a | 45.51a | 7.87b |
| 1 mM | 14.06a | 68.42a | 46.40a | 5.58c |
| Drought stress | | | | |
| 0 (control) | 13.97a | 74.97a | 54.80a | 6.44c |
| 30% | 13.10a | 64.93ab | 40.88b | 7.40b |
| 60% | 11.56b | 57.86b | 34.17c | 10.58a |

The same letters in each column indicate no significant difference at the 5% probability level in the Duncan test. LAI, CHLI, RWC, EC were the abbreviations of Leaf area index, Chlorophyll index, Relative water content, Electrical conductivity

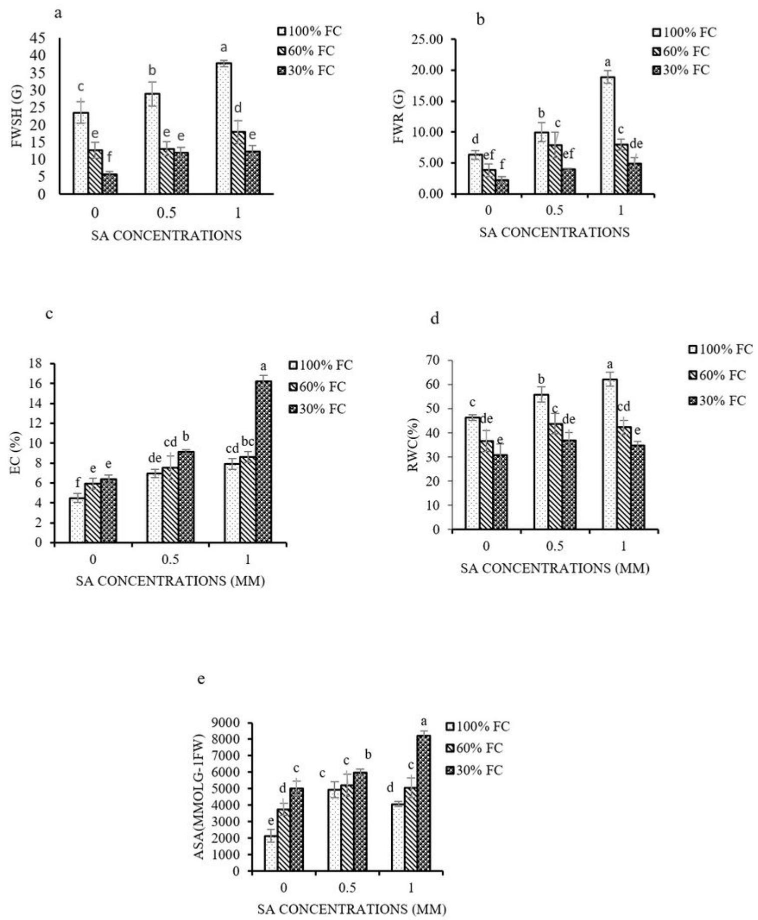
Table 5

Mean comparison of salicylic acid and drought stress effects on morphological parameters of pepper plants

| Treatments | AC (%) | TPC (mg of GAE $g^{-1}$FW) | ASA(μmol$g^{-1}$FW) | PPO (unit $g^{-1}min^{-1}$) | APX (unit $mg^{-1}$protein) |
|---|---|---|---|---|---|
| Ascorbic acid | | | | | |
| 0mM (control) | 15.49c | 1.76c | 3.63b | 37.88c | 28.86c |
| 0.5 mM | 37.99b | 4.22b | 5.38a | 44b | 54.93b |
| 1 mM | 47.14a | 5.48a | 5.77a | 59.83a | 77.83a |
| Drought stress | | | | | |
| 0 (control) | 26.40c | 3.32b | 3.72c | 39.07c | 42.83c |
| 30% | 32.27b | 3.45b | 4.67b | 47.85b | 53.94b |
| 60% | 41.95a | 4.69a | 6.40a | 54.79a | 64.85a |

The same letters in each column indicate no significant difference at the 5% probability level in the Duncan test. AC, TPC, ASA, PPO, and APX were the abbreviations of Antioxidant capacity, Total phenol content, Ascorbic acid, Polyphenol oxidase and Ascorbate peroxidase.

## Figures




## Figure 1

Effects of foliar application of SA and drought stress on some factors pepper plants. FWSH, DWR, EC, RWC and AsA were the abbreviations of Fresh weight shoot, Dry weight root, Electrical Conductivity, Relative water content and ascorbic acid.

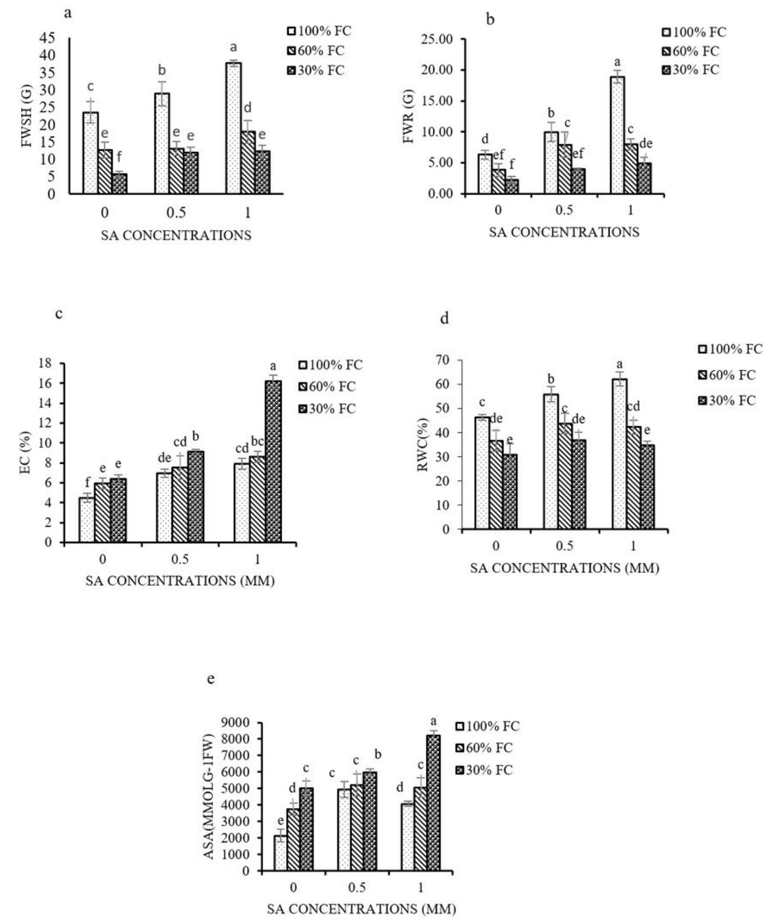



**Figure 1**

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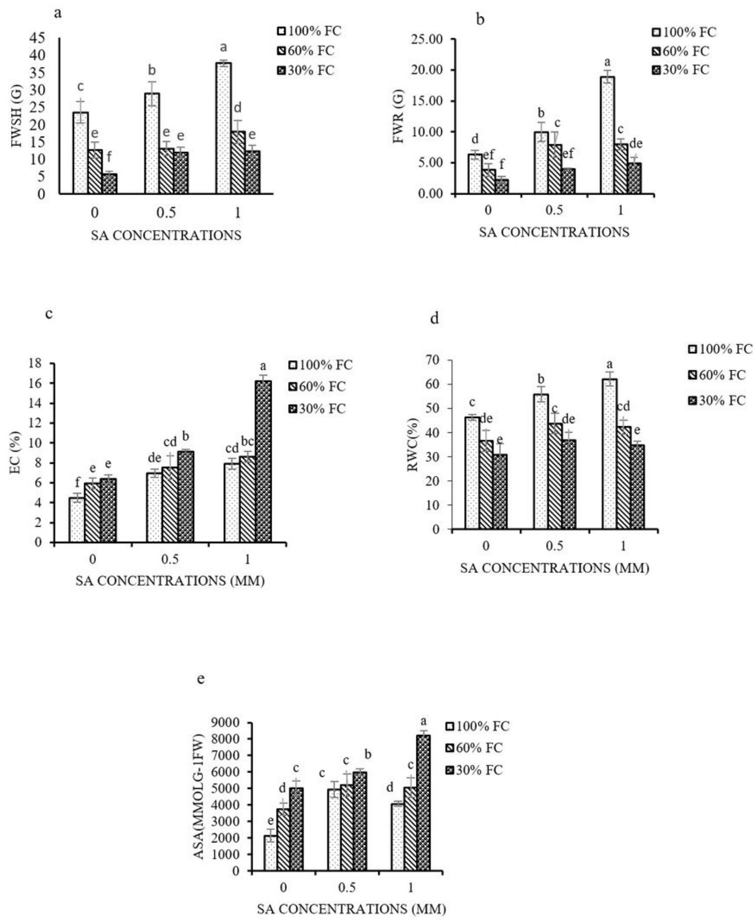


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Effects of foliar application of SA and drought stress on some factors pepper plants. FWSH, DWR, EC, RWC and AsA were the abbreviations of Fresh weight shoot, Dry weight root, Electrical Conductivity, Relative water content and ascorbic acid.

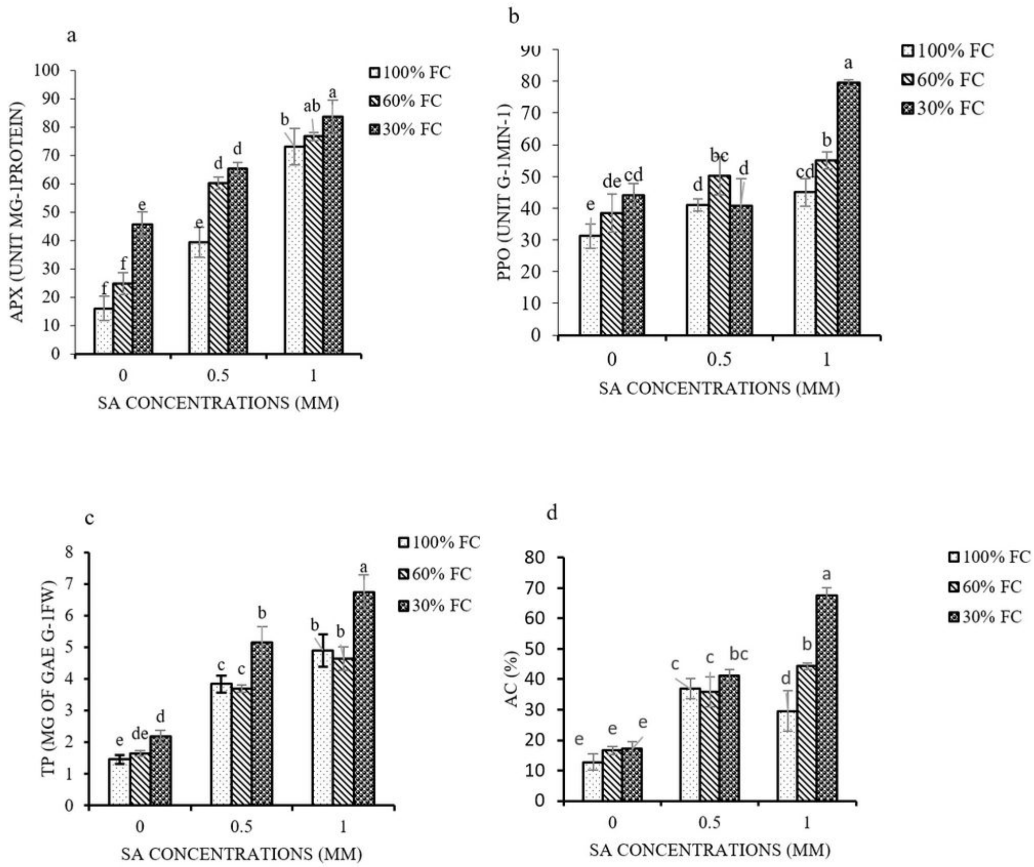
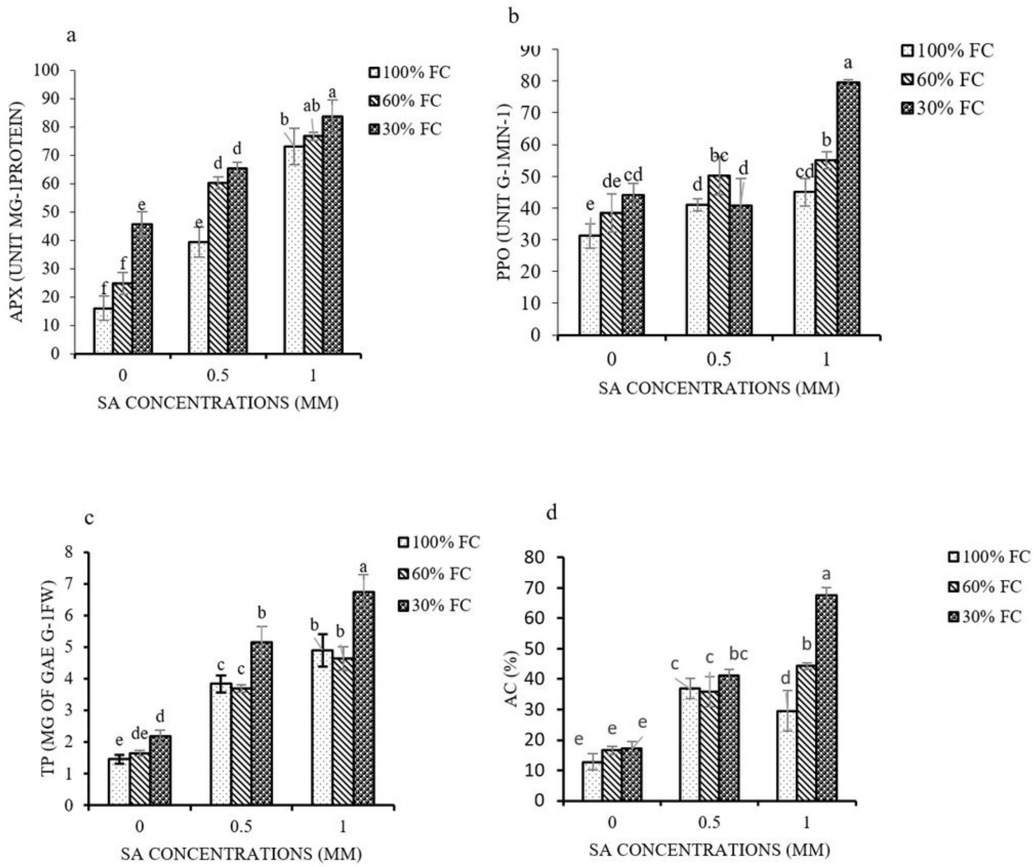



Figure 2

Effects of foliar application of SA and drought stress on some factors pepper plants. PPO, APX, TP and AC were the abbreviations of Polyphenol oxidase, Ascorbate peroxidas, Total phenol content and Antioxidant capacity

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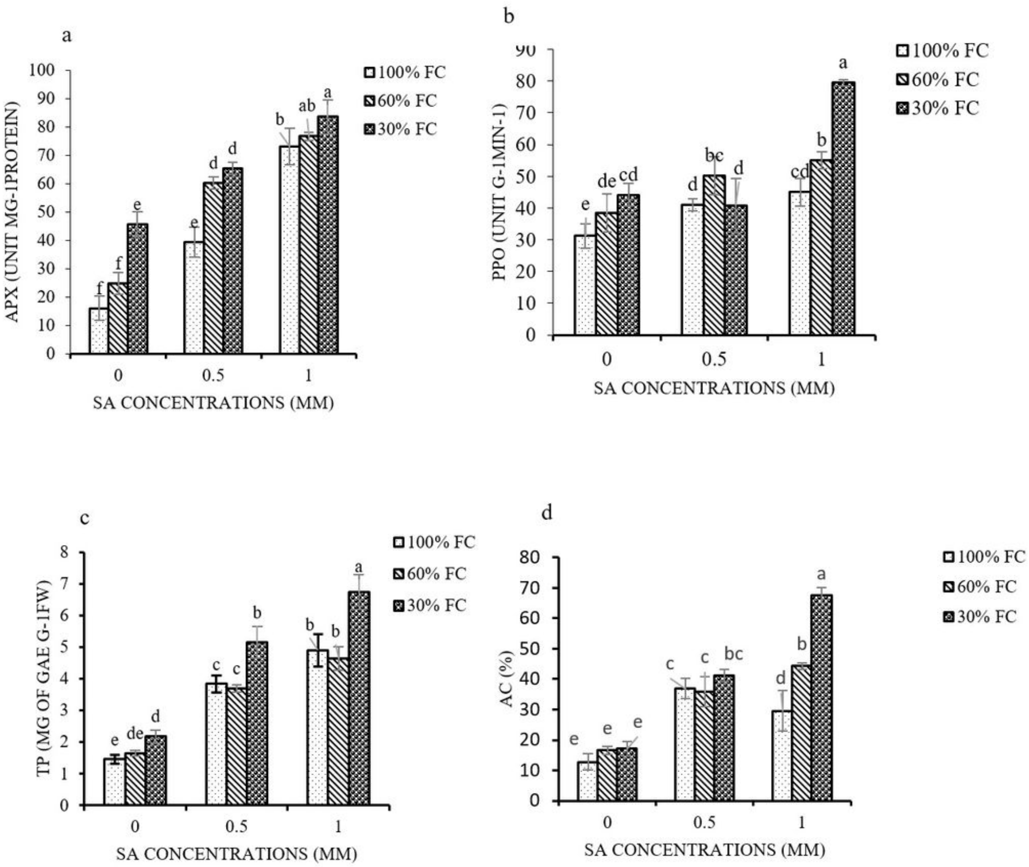


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