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Assessing the Impact of Sodium Nitroprusside Foliar Application on Drought Tolerance of Henna (Lawsoniainermis L.) Ecotypes

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ABSTRACT

Objective: This study aimed to investigate the effect of sodium nitroprusside foliar application (SNP) on the performance of henna (Lawsoniainermis L.) ecotypes under drought stress conditions.

Methods: The experiment was conducted using a completely randomized design with a factorial arrangement. The factors included three henna ecotypes (Shahdad, Bam andRudbar-e-Jonub), three levels of irrigation (100, 75 and 50% of field capacity) and spraying (control and sodium nitroprusside). Various growth and physiological parameters were measured to assess the performance of henna under different treatments.

Results:With a decrease in water level, the amount of photosynthetic pigments, growth indices, net photosynthesis, and ultimately leaf dry matter yield decreased. However, the application of sodium nitroprusside spray treatment was able to significantly increase leaf surface index, product growth rate, net photosynthesis, and leaf dry matter yield compared to the control treatment at irrigation levels of 75% and 50% of the agricultural capacity. Among them, the highest percentage increase in the measured traits was related to the Shahdad genotype.

Conclusions: This study provides valuable insights into the potential of SNP foliar application in enhancing the performance of henna plants under drought stress conditions. The findings highlight the importance of selecting suitable henna ecotypes for cultivation in regions prone to drought stress. Additionally, the study contributes to the limited literature on the use of SNP as a potential tool for improving plant tolerance to drought stress.

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1- Introduction

In general, the cultivated area of medicinal plants in the country is reported to be approximately 39,427 hectares, with a production yield of about 100,268 tons. The highest cultivated area of medicinal plants after cumin (with an area of about 18,000 hectares) is related to the henna plant, with an area of 7,944 hectares (Agricultural Statistics, 2014).

Henna is a native plant of North Africa and South Asia, extensively used in religious ceremonies of Hindu and Muslim communities in South Asian countries such as India, Iran, Pakistan, and the United Arab Emirates (Kumar Singh *et al*., 2015). This plant is a perennial shrub that is cultivated in tropical regions for its ornamental qualities and the dye obtained from its color. Henna is a bushy plant with numerous branches and without thorns, reaching a height of up to 4 meters. Its leaves are small, measuring approximately 1.5 to 5 centimeters in length and 0.5 to 2 centimeters in width. The leaves are oval-shaped, pointed, serrated, and inclined towards green to dark brown on the branches. Henna branches have a square cross-section and are green in color, turning red with age. The flowers of this plant are large clusters with numerous small and fragrant flowers. The fruit of the henna plant is a brown capsule that opens irregularly when ripe, dividing into four parts. Each plant produces a large number of seeds. Henna seeds are pyramidal, smooth, hard, and brownish in color, measuring 2 millimeters in size (Chaudhary *et al.*, 2010).

Phenological studies have shown that henna sprouts between 12 to 14 days after sowing the seeds. Flowering in the plant begins approximately 90 days after planting and continues continuously. Flowering slows down during the hot months (July and August) but accelerates again towards the end of the growth period with cooler weather. Complete fruit ripening in different regions occurs from late September to late November (Pasandi Pour *et al*., 2018).

The number of harvests is influenced by the planting date, irrigation frequency, and the age of the plant, ranging from two to three harvests. The first henna harvest is the most valuable and superior compared to subsequent harvests. The harvesting time of henna varies depending on the climate and the number of harvests, usually taking place from July to November. The total growth period of the plant ranges from 190 to 220 days in the studied regions (Pasandi Pour *et al.*, 2018).

The henna plant's product is a natural and non-toxic substance, and considering its other advantages such as affordability and availability, it can be a real opportunity for the treatment and prevention of skin infections, especially for developing countries (Behdani*et al*., 2009). This plant has antibacterial effects, especially against gram-positive bacteria. It also has antifungal effects against fungi such as Trichophyton, Sporotrichum, and Cryptococcus. These antibacterial and antifungal effects are attributed to lawsone, a compound found in henna (Al-Rubiay*et al*., 2008). Another beneficial property of henna is its anticancer effect. Chloroform extract of henna has a strong cytotoxic effect on liver and breast cancer cells and inhibits the growth of cancer cells by suppressing the Myc-c gene, which plays a crucial role in cell growth (Endrini*et al*., 2007). Henna is also used in traditional medicine for the treatment of various diseases such as menstrual disorders, edema, rheumatism, bronchitis, and hemorrhoids (Bich *et al.*, 2004).

Henna leaves contain a coloring substance called lawsone or 2-hydroxy-1,4-naphthoquinone, multiple phenolic glycosides, coumarins, xanthones, quinoids, glycosides, beta-sitosterol, flavonoids such as luteolin, 6% fats, 2-3% resin, 7-8% tannins, and 1.2% essential oil (Trease and Evans, 1996).

The cultivation of henna plants in Kerman province is limited to the cities of Bam, Shahdad, Kerman, and Rudbar-e-Jonub. The cultivated area in these regions ranges from a minimum of 3 hectares with an average yield of 1.4 tons per hectare in Shahdad to a maximum of 7,500 hectares with an average yield of 5.6 tons per hectare in Rudbar-e-Jonub (Pasandi Pour *et al.*, 2018). The highest distribution of henna cultivation (98.12%) is reported in the elevation range of 450 to 511 meters above sea level, while the lowest distribution (87.1%) is reported at an elevation of 1,080 meters above sea level (Pasandi Pour *et al.*, 2018). The lower limit of henna cultivation in terms of elevation is 450 meters above sea level (in Shahdad), and the upper limit is 1,080 meters above sea level (in Bam). The annual rainfall in the henna cultivation areas in Kerman province ranges from 28.35 to 143.5 millimeters, with the highest and lowest rainfall recorded in the regions of Kermanouj and Shahdad, respectively. The average relative humidity for these areas is 33.6%, and the average annual temperature is 26.5 degrees Celsius (Pasandi Pour *et al.*, 2018).

Limited studies have been conducted on the ecophysiological needs of henna plants. In this regard, the effect of plant density on growth and yield indices of henna ecotypes in Shahdad has been reported, with the highest dry leaf yield obtained from Shahdad and Rudbar-e-Jonub ecotypes (Pasandi Pour and Farahbakhsh, 2018). A study conducted on henna plants in Kerman city's climatic conditions showed that although the Shahdad ecotype had a significant advantage in relative growth rate and product growth rate compared to the Bam and Rudbar-e-Jonub ecotypes, there was no significant difference in the total dry weight and leaf dry weight among these ecotypes (Pasandi Pour and Farahbakhsh, 2018).

Plants are continuously affected by unfavorable environmental factors during their growth period. Some of these unfavorable factors, such as water stress, limit the growth and development of plants (Azizi Nia *et al.*, 2005). Water deficiency affects cellular osmotic pressure, resulting in the opening and closing of stomata, photosynthesis, respiration, and transpiration processes. It also affects enzyme processes directly controlled by water potential, resulting in negative effects on plant growth. Numerous reports have shown the impact of water deficiency, from mild to severe stress, on the disruption of physiological processes in plants, changes in carbohydrate and nitrogen metabolism, protein structure, and enzyme activity (Brar et al., 1990). Drought stress induces oxidative stress in plants, leading to an increase in reactive oxygen species (ROS). Under these conditions, the activity of antioxidant enzymes such as superoxide dismutase, peroxidase, and catalase increases (Hayat and Ahmad, 2007). Lieutenant and colleagues (2017) demonstrated in their investigation on the fenugreek plant that under drought stress conditions, photosynthesis and the level of photosynthetic activity decreased, resulting in a shorter seed filling period and reduced yield. In an experiment on the basil plant, Ramroudi and Khomar (2013) showed that drought stress reduced the chlorophyll content of basil. Abuftileh Nejad and colleagues (2013) demonstrated in a study on different varieties of mung bean that drought stress led to a decrease in chlorophyll content. In a study on red kidney bean plants, Lahuti and colleagues (2013) showed that drought stress caused a decrease in leaf chlorophyll content. Results from a study on black cumin showed that drought stress reduced cell division and cell size, leading to a decrease in plant height (Rezaei Chiane and Pirzad, 2013). Drought stress leads to a reduction in dry matter in all parts of the plant, although some organs show different degrees of dry matter reduction. For example, drought stress reduces the dry weight of aerial organs and flowers (Asrar and Elhindi, 2011). The occurrence of drought stress during various growth stages, especially the reproductive stage, results in a reduction in the duration of photosynthetic activity, the transfer of photosynthates to the seeds, the retranslocation of stored stem reserves to the seeds, and ultimately, a decrease in seed yield in fennel plants (Pouryousef, 2012). In an experiment on cumin plants, it was shown that drought stress during the flowering stage had the greatest impact on reducing yield components and cumin yield (HedidiMasouleh and colleagues, 1398). Another study on the common bean plant revealed that increasing the concentration of salicylic acid increased the levels of photosynthetic pigments, especially chlorophyll, and cell division, leading to an increase in plant height (Shokari and colleagues, 2015). Laspeina and colleagues (2005) reported that pre-treatment of sunflower plants with 0.5 mM sodium nitroprusside under stress conditions resulted in better growth compared to untreated plants. Symptoms such as chlorosis and necrotic spots on leaves, reduction in chlorophyll and relative leaf water content decreased with the application of this substance. In a study conducted by Farouk and colleagues (2009), it was found that drought stress significantly reduced seedling growth in rice, while treatment with sodium nitroprusside improved plant growth. According to their belief, the external application of nitric oxide significantly increased water uptake in plants under water stress. Nile and colleagues (2008) reported that the external application of sodium nitroprusside stimulated stomatal closure and protected cells against oxidative stress. The solution of this compound reduced membrane permeability, electrolyte leakage, and the level of H_2O_2 in the leaves (Neill *et al*., 2008).

In a study on wheat plants, it was shown that the external application of sodium nitroprusside stimulated stomatal closure and protected cells against oxidative stress. They also stated that the foliar application of this compound reduced membrane permeability, electrolyte leakage, and the level of H_2O_2 in the leaves (Neill *et al*., 2008). The results showed that sodium nitroprusside to a great extent mitigated the adverse effects of stress on basil plants by improving the plant's enzymatic system and removing free radicals, leading to improved plant growth under stress conditions (Asghari *et al*., 2020). The use of sodium nitroprusside resulted in an increase in the number of lateral branches, thousand seed weight, seed yield, fruit diameter, and weight in the paper-skinned pumpkin plant (Yadollahi*et al*., 2018). An experiment on the mint plant showed that the foliar application of sodium nitroprusside increased chlorophyll content, essential oil percentage, and antioxidant activity (GorginiShabankareh and Khorasani Nejad, 2017). Researchers reported that the application of sodium nitroprusside delayed the senescence of tomato leaves by preventing the degradation of chlorophyll and soluble proteins, especially rubisco (Shehab *et al*., 2010). In a study on the mint plant, it was shown that the application of sodium nitroprusside increased chlorophyll a, b, and total chlorophyll content, demonstrating the protective and regulatory role of sodium nitroprusside against drought stress (GorginiShabankareh and Khorasani Nejad, 2017). Moghadam and colleagues (2017) stated that the application of sodium nitroprusside could be beneficial in reducing the adverse effects of water stress on the pennyroyal medicinal plant.

Understanding the ecological requirements of henna is essential for the development of its cultivation. Therefore, it is necessary to study the characteristics of this species further. In this research, we attempted to create a basis for the optimal management of henna cultivation in Kerman province by investigating the effects of drought stress and the foliar application of sodium nitroprusside on common henna ecotypes in Kerman province.

2-Materials and Methods

2-1-Description of the study area

Shahdad is located 87 kilometers northeast of the city of Kerman, with coordinates of 57 degrees and 42 minutes longitude and 30 degrees and 25 minutes latitude. It is situated at an altitude of 450 meters above sea level. The average annual rainfall in this city is 28.35 millimeters, the average annual temperature is 27.47 degrees Celsius, and the relative humidity is 21.84 percent.(Figure 1)

Figure. 1 *Geographical location of Shahdad*

2-2-Data collection and analysis

The experiment was conducted in a split factorial design based on a complete random block design with three replications. The main factor in this experiment included three levels of drought stress (100%, 75%, and 50% of field capacity), and the sub-factors included three henna ecotypes (Robar, Shahdad, and Bam) and sodium nitroprusside foliar spray (application and non-application). The drought stress treatments started after the complete establishment of the plants (at a height of approximately 10 cm) and continued until the end of the growth season. The sodium nitroprusside foliar spray was applied in three occasions (one week before the start of stress, simultaneously with the start of stress, and one week after the start of stress). The planting was done on April 4th. Each plot had a length of 2 meters and a width of 3 meters. Manual weed control was performed throughout all growth stages. Irrigation was done by flooding using a volumetric meter. After planting until the plants turned green and established, irrigation was carried out completely.

To measure the leaf area index and growth rate of the crop, sampling was done in 6 stages at 10-day intervals, with 6 samples extracted from each plot while considering the edge effect. The leaf area was measured using a leaf area meter. To measure the dry weight of the samples, they were individually placed in paper bags and kept in an oven at a temperature of 75 degrees Celsius for 24 to 48 hours. The leaf area index and growth rate were calculated using the following formulas.

(Formula 1)

$$\mathrm{LAI} = \frac{1}{\mathrm{GA}} \times \frac{\mathrm{LA}_2 + \mathrm{LA}_1}{2}$$

(Formula 2)

$$\mathrm{CGR} = \frac{1}{\mathrm{GA}} \times \frac{\mathrm{W}_2 - \mathrm{W}_1}{\mathrm{T}_2 - \mathrm{T}_1}$$

LA : leaf areaGA : ground area

W : shoot weightT :Time

The Lichtenthaler method was used to measure the amount of chlorophyll and carotenoids (Lichtenthaler, 1987). Fresh plant leaves weighing 1.0 gram were ground in a mortar containing 15 mL of 80% acetone, and the absorption was measured using a spectrophotometer SCO-TECH model SPUV-26 at wavelengths of 646.8 nm, 663.2 nm, and 470 nm.

The catalase activity was measured based on the decrease in oxygen absorption at a wavelength of 240 nm (Dhindsa et al., 1981). The reaction mixture (3 mL) consisted of 50 mM potassium phosphate buffer (pH 7), 15 mM hydrogen peroxide, and 100 µL of enzyme extract. By adding hydrogen peroxide to the reaction mixture, the reaction started, and the decrease in oxygen absorption was measured at 240 nm using a spectrophotometer for 30 seconds.

For protein concentration measurement, 100 µL of enzyme extract was added to test tubes, followed by the addition of 5 mL of Bradford reagent and vigorous vortexing. After 25 minutes, the absorption was measured at 595 nm using a spectrophotometer (Bradford, 1976).

The proline content was measured using the method of Bates et al. (1973). 0.2 g of frozen plant tissue was homogenized in 10 mL of 3% sulfosalicylic acid solution, and the resulting extract was centrifuged at 10,000 rpm for 5 minutes. Then, 2 mL of the supernatant was mixed with 2 mL of ninhydrin reagent and 2 mL of acetic acid, and the mixture was heated at 100°C in a water bath for one hour. Immediately, the tubes containing the mixture were cooled in an ice bath. At this stage, 4 mL of toluene was added to the mixture, and the tubes were vigorously shaken. After keeping the tubes stationary for 15 to 20 seconds, two separate layers were formed. The absorption of the upper layer containing toluene and proline was measured at 520 nm.

In this study, net photosynthesis, stomatal conductance, and transpiration during early flowering were measured using a CI-340 photosynthesis meter manufactured by CID Bio-Science, USA. For this purpose, five plants from the middle row of each experimental plot were selected. Measurements were performed using the terminal leaves of the plants between 11 am and 2 pm in one session.

In mid-November, the plants were harvested from a 5 cm soil surface and dried in a ventilated oven at 75°C for 48 hours to calculate the dry leaf yield.

2-3- Data analysis

The SAS v. 9.1 software was used for variance analysis, and the means were compared using the MSTATC software. Mean comparisons were performed using the Duncan's test at a significance level of 5%.

3-Results and Discussion

3-1- Leaf surface index

According to the results of the analysis of variance, the simple effects of drought stress, ecotype, and foliar spraying, as well as the interaction effect of irrigation in the ecotype, were significant at the 1% level for this trait (Table 1). The comparison of the mean effect of foliar spraying showed that the highest leaf surface index (11.2) was obtained from the treatment of sodium nitroprusside foliar spraying, which resulted in an 8% increase in this trait compared to the control treatment (Table 5). The comparison of the mean effect of irrigation interaction in the ecotype showed that under normal irrigation conditions and irrigation at 50% of the farm's irrigation capacity, the highest leaf surface level was related to the Shahdad ecotype (Table 6).

Yari *et al*. (2014) reported that drought stress caused a significant decrease in the leaf surface index of spring safflower. The decrease in leaf surface under dry conditions can be considered an unavoidable event (Torrecillas et al., 2003). This approach minimizes water loss when stomata are closed and occurs in many species under stress conditions (Ruiz-Sanchez *et al.*, 2000).

3-2- Crop growth rate

This trait was significantly affected by the main effects of irrigation, ecotype, and foliar spraying, as well as the interaction effect of irrigation in foliar spraying at the 1% probability level (Table 1). Among the studied ecotypes of henna, the highest and lowest crop growth rates were related to the Bam and RoudbarJonoub ecotypes, respectively, with a significant difference between them (Table 4).

The comparison of the mean effect of irrigation interaction in foliar spraying showed that the application of sodium nitroprusside foliar spraying resulted in a significant increase in crop growth rate compared to the control treatment at each irrigation level applied (Table 7). These results also showed that the highest crop growth rate (91.11 grams per square meter per day) was achieved in the normal irrigation treatment accompanied by sodium nitroprusside foliar spraying (Table 7).

It seems that the reason for the decrease in crop growth rate under drought stress conditions is the reduction in leaf area and number, disruption in photosynthesis, and decrease in dry matter production. Under drought stress conditions, the decrease in leaf water potential also leads to a decrease in crop growth rate, which is due to the increase in respiration rate accompanied by an increase in plant temperature and a decrease in photosynthesis. On the other hand, changes in leaf surface, which are an important factor in carbon assimilation, cause variations in crop growth rate under drought stress conditions.

3-3-Pure photosynthesis

Photosynthesis is the main determining factor of plant growth and performance, and its preservation under environmental stress conditions is crucial for maintaining performance stability (Javadipour et al., 2013). The effects of different irrigation treatments, foliar spraying, and genotype interaction of irrigation and foliar spraying on net photosynthesis were found to be statistically significant (Table 1). According to the results, the highest and lowest mean values of net photosynthesis were 69.28 and 58.14 micromoles per square meter per second, respectively, for the treatment of irrigation based on 100% field capacity and genotype Shahdad, and the treatment of irrigation based on 50% field capacity and genotype RoudbarJonoub (Table 6). The comparison of mean values showed that the highest net photosynthesis was related to the treatment of foliar spraying with sodium nitroprusside at irrigation levels of 75% and 50% of field capacity (Table 7). The decrease in photosynthesis can be attributed to the reduction in stomatal conductance, which was also observed under stress in this study. Although stomatal closure under stress conditions is done to reduce water loss, it can reduce photosynthesis below the compensatory point by preventing the entry of carbon dioxide (Ashraf and Harris, 2004).

4-4-Transpiration rate

According to the results of the analysis of variance, the main effects of drought stress, ecotype, and foliar application were found to be significant for this trait at the 1% level (Table 1). Reducing the irrigation water amount from 100 to 75 and 50 percent of the field capacity resulted in a significant decrease in transpiration rate in this plant (Table 3). The comparison of the mean effects of ecotype showed that the highest transpiration rate (21.6 millimoles per square meter per second) was obtained from the Shahdad ecotype, which had a significant difference compared to the Bam and RoodbarJonub ecotypes (Table 4). The comparison of the mean effects of foliar application showed that the use of sodium nitroprusside resulted in an 11% increase in transpiration rate compared to the control treatment (Table 5). Transpiration rate is influenced by factors such as vapor pressure difference, water flow resistance, and the ability of the plant and soil to transport water to the transpiration site. Additionally, solar radiation, temperature, relative humidity, and wind are among the other environmental factors that affect transpiration (Gardner et al., 1985). The reduction in transpiration rate in marigold plants under drought stress conditions has been reported by Zafari et al. (2017).

3-5-Stomatal conductance

Stomata are the main entry points of the plant and the opening and closing of stomata play a crucial role in gas exchange, transpiration, and photosynthesis. The size of stomata usually changes in response to environmental and internal factors, which leads to variations in transpiration and stomatal conductance (Condon et al., 2004). According to the results of the analysis of variance, the main effects of drought stress and foliar application at the 1% level and the interaction effect of irrigation and ecotype at the 5% probability level were found to be significant for this trait (Table 1). The comparison of the mean effects of foliar application showed that the highest stomatal conductance (205 millimoles per square meter per second) was obtained from the sodium nitroprusside treatment, which had a 10% increase compared to the control treatment (Table 5). The comparison of the mean effects of irrigation and ecotype showed that under normal irrigation conditions (100% field capacity), there was no significant difference in stomatal conductance among the studied ecotypes. However, under 50% field capacity irrigation, the Shahdad ecotype had the highest stomatal conductance (156 millimoles per square meter per second) (Table 6).

3-6-Photosynthetic pigments

The analysis of variance results showed that the interaction effect of irrigation and foliar application was significant at the 1% probability level for the total chlorophyll content, chlorophyll a, chlorophyll b, and carotenoid content (Table 2). The comparison of the mean effects of irrigation and foliar application (Table 7) showed that under normal irrigation conditions (100% field capacity), there was no significant difference in photosynthetic pigments between the control and foliar application treatments. However, at 75% and 50% field capacity irrigation levels, the foliar application of sodium nitroprusside significantly increased the photosynthetic pigments compared to the control treatment (Table 7).

In this study, a decrease in chlorophyll content was observed under drought stress. Consistent with the results of this study, a reduction in chlorophyll content has been reported in other medicinal plants such as Mentha pulegium (Abaspour and Rezaei, 2014), Coriandrum sativum (Noorzad et al., 2015), Nigella sativa (Kabiri et al., 2014), and Foeniculum vulgare (Setayeshmehr and Ganjalikhani, 2013). Recent studies have shown that under drought stress conditions, both stomatal closure and non-stomatal limitations (such as oxidative damage to chloroplasts) lead to a decrease in photosynthesis. The reduction in photosynthetic pigments under drought stress conditions may be due to the destruction of chloroplast structure and photosynthetic apparatus, chlorophyll oxidation reactions with singlet oxygen, the degradation of chlorophyll synthesis precursors, and the activation of chlorophyll-degrading enzymes, including chlorophyllase (El-Tayeb, 2005; Neocleous and Nasilakakis, 2007).

According to the results of this study, a decrease in carotenoid content was reported in Nigella sativa (Kabiri et al., 2014) and Foeniculum vulgare (Setayeshmehr and Ganjalikhani, 2013) under drought stress. The decrease in carotenoids under drought stress conditions can be attributed to the production of singlet oxygen in thylakoids. Carotenoids are capable of absorbing high-energy short-wavelength light and converting singlet oxygen into triplet oxygen, thus exhibiting their antioxidant role (Abaspour and Rezaei, 2014).

3-7-Catalase

The simple effects of irrigation treatments and foliar spraying, as well as their interactive effects, were found to be significant for this trait at a 1% probability level (Table 2). According to the results of the comparison of the mean values of the highest and lowest catalase activity levels with values of 0.468 and 0.152 milligrams per gram of protein, respectively, these values were associated with irrigation treatment based on 50% of the soil field capacity without foliar spraying and irrigation treatment based on 100% of the soil field capacity with sodium nitroprusside foliar spraying (Table 7). Furthermore, these results indicated that the highest level of antioxidant activity for catalase was associated with the treatment without sodium nitroprusside foliar spraying at the irrigation levels of 75% and 50% of the soil field capacity.

In this study, the activity of the antioxidant enzyme catalase increased in response to drought stress. According to the results of previous studies on black cumin (Ahmadpour Dehkordi and Balouchi, 2012), safflower (Bayan et al., 2013), and turmeric (Zamani *et al.,* 2012), an increase in the activity of this antioxidant enzyme has been reported under drought stress conditions.

Under drought stress, where the CO_2 uptake decreases due to the closure of stomata, the internal energy increases, the electron transfer capacity of photosynthesis shifts towards accumulation, and as a result, an increase in ROS concentration occurs, leading to lipid peroxidation, protein degradation, and DNA oxidation. It is at this point that oxidative enzymes (such as catalase in this study) become more active.

Catalase is an enzyme involved in hydrogen peroxide detoxification, therefore, an increase in the activity of this enzyme leads to the removal of hydrogen peroxide by breaking it down into water and oxygen. Although hydrogen peroxide is toxic at high concentrations, it can act as a signaling molecule in low concentrations and activate resistance-related genes in plants through the ascorbate-glutathione antioxidant system (Unyayar*et al.*, 2005). Induction of catalase activity helps to overcome oxidative stress by detoxifying hydrogen peroxide and protects proteins, nucleic acids, and lipids against ROS compounds (Rastgoo and Alemzadeh, 2011).

3-8-Proline

According to the results of the analysis of variance, only the simple effects of drought stress and foliar spraying were found to be significant for this trait at a 1% probability level (Table 2). The highest (0.7946 milligrams per gram fresh weight leaf) and lowest (0.2682 milligrams per gram fresh weight leaf) proline levels were obtained from irrigation treatments based on 50% and 100% of the soil field capacity, respectively (Table 3). The comparison of the mean effect of foliar spraying showed that the highest proline level (0.734 milligrams per gram fresh weight leaf) was obtained from the sodium nitroprusside foliar spraying treatment, which resulted in a 10% increase compared to the control treatment (Table 5).

Based on the obtained results, it can be stated that the plant tries to maintain its osmotic pressure in response to drought stress, and it does so by increasing osmolytes such as proline and soluble sugars, which help maintain cell pressure and turgor. Proline accumulation in plant cells is considered to be involved in mechanisms of drought resistance (Yin *et al*., 2009). High proline levels enable the plant to maintain its water potential (Valliyodan and Nguyen, 2006). High proline levels reduce the levels of free radicals in response to osmotic stress and improve plant performance. It has been found in many studies that proline, as an osmolyte, regulates plant biosynthesis and moderates oxidative stress caused by free radicals (Hong *et al.,* 2003). Increased proline content during drought stress indicates its role in regulating osmotic pressure (Ashraf and Foolad, 2007). Increased proline content under drought stress has been reported in pumpkin (Rabi Anguráni*et al.*, 2017), peanut (Smith et al., 2002), wheat (Hong-Bo *et al*., 2006), and sugar beet (Monreal *et al.*, 2007).

3-9-Protein

Only the simple effect of irrigation treatment was found to be statistically significant for this trait (Table 2). The results of the comparison of the mean effect of irrigation treatment showed that reducing the irrigation water from 100% to 75% did not result in a significant change in protein content, but reducing the irrigation water to 50% of the soil field capacity led to a 30% decrease in protein content compared to the control treatment (Table 3).

The results of this study indicated a significant reduction in total protein content under drought stress. This is consistent with the results of studies on basil (SalarpourGhoraba and Farahbakhsh, 2014), black cumin (Rezaei Chiyaneh and Pirzad, 2014 and Kabiri *et al.,* 2014), and chicory (Sodaeizadeh and Mansouri, 2014) under drought stress conditions. Non-biological stresses inhibit the synthesis of certain proteins and stimulate the production of others, although the overall trend is towards a decrease in total protein levels (Ericson and Alfinito, 1984). Drought stress induces the expression of intracellular protease genes, leading to protein degradation and nitrogen mobilization, followed by the synthesis of compatible solutes. Therefore, the reduction in protein content under drought stress is associated with a decrease in protein synthesis and an increase in the activity of protein-degrading enzymes (Feller, 2004). Oxidative stress can also be one of the reasons for the decrease in protein content. The production of reactive oxygen species such as superoxide radicals or hydroxyl radicals leads to the oxidation of amino acids and serious damage to the structure and function of proteins, resulting in a decrease in protein content (Amini and Haddad, 2013). In this study, with the increasing intensity of drought stress, the total soluble protein content in the leaf decreased, which was accompanied by a significant increase in catalase activity. Therefore, the significant increase in catalase activity and the significant decrease in protein content in the leaf under severe drought stress can be attributed to both protein degradation and reduced protein synthesis.

3-10-Leaf Dry Weight

According to the results of the analysis of variance, the simple effects of drought stress, foliar spraying, and their interaction were found to be significant for this trait at a 1% probability level (Table1).The comparison of the mean effect of foliar spraying showed that the highest leaf dry weight (402 grams per square meter) was obtained from the sodium nitroprusside foliar spraying treatment, which resulted in an 8.9% increase compared to the control treatment (Table5).The comparison of the mean effect of irrigation showed that although the highest leaf dry weight was obtained from the Shahdad ecotype, there was no significant difference in leaf dry weight among the studied ecotypes at the irrigation levels of 100% and 50% of the soil field capacity. However, the highest leaf dry weight (405 grams per square meter) was obtained from the Bam ecotype at the irrigation level of 75% of the soil field capacity (Table6).

The results of this study showed a significant reduction in leaf dry matter production under drought stress. This is consistent with the results of studies on Norouzak (Dashti *et al.,* 2015), sorghum (Omidvar *et al.,* 2015), black cumin (Rezaei Chiyaneh and Pirzad, 2014 and Kabiri et al., 2014), tuberose (Sodaeizadeh and Mansouri, 2014), coriander (Nourzadeh*et al.,* 2014), peppermint (Shahriari et al., 2013), and anise (Mohammadi Alborzi *et al.,* 2012). Naturally, water deficiency and consequently decreased osmotic pressure within the cell, as well as reduced nutrient uptake due to increased soil osmotic potential, lead to a decrease in cell size and leaf growth. As a result, with the reduction in leaf surface area, the absorption of sunlight and consequently plant photosynthesis decreases, leading to a reduction in dry matter production.

Table 1- Variance analysis of measured traits of Henna

Sources Of Variation	Degree Of Freedom	Leaf Yield Index	Growth Rate	Net Photosynthesis	Transpiration Rate	Stomatal Conductance	Leaf Dry Yield
Block	2	0.054	0.421	0.0185	63.72	1.779	1181
Irrigation	2	10.33**	61.29**	64.991**	48868**	369.4**	217199**
Error a	4	0.0300	0.143	0.170	45.55	0.276	2630
Ecotype	2	0.286**	2.798**	3.1602**	1374**	2.527 ns	1558 ns
Foliar spraying	1	0.428**	12.83**	4.746**	4968**	32.838**	17413**
Irrigation in ecotype	4	0.310**	0.287 ns	1.761**	406.88 ns	8.149*	7920**
Irrigation in foliar spraying	2	0.056 ns	0.889**	0.6185*	595.12 ns	2.588 ns	1810 ns
Ecotype in decay	2	0.0094 ns	0.113 ns	0.073 ns	370.35 ns	0.495 ns	293.19 ns
Irrigation in ecotype in foliar spraying	4	0.0127 ns	0.103 ns	0.143 ns	356.35 ns	0.598 ns	364.07 ns
Error	30	0.02083	0.131	0.135	198	2.560	742
Coefficient of variation(%)	-	7.11	3.82	6.4	7.2	10.23	7

ns, ** and *: non significant and significant at the 1% and 5% probability levels, respectively

Table 2- Variance analysis of measured traits of Henna

Sources Of Variation	Degree Of Freedom	Chlorophyll a	Chlorophyll b	Chlorophyll Total	Carotenoid	Catalase	Proline	Protein
Block	2	0.00005	0.00067	0.00002	0.0019	2.952	0.000005	0.0123
Irrigation	2	0.040**	0.374**	0.0107**	0.2949**	3406.9**	0.00018**	0.651**
Error a	4	0.00008	0.0011	0.000006	0.00041	1.345	0.000003	0.0170
Ecotype	2	0.00015 ns	0.0013 ns	0.00005 ns	0.00026 ns	0.4964 ns	0.000002 ns	0.0005 ns
Foliar spraying	1	0.0092**	0.0852**	0.0024**	0.0399**	90.016**	0.00003**	0.0439 ns
Irrigation in ecotype	4	0.000072 ns	0.0006 ns	0.00001 ns	0.00047 ns	2.056 ns	0.0000004ns	0.0019 ns
Irrigation in foliar spraying	2	0.0016**	0.0163**	0.00053**	0.01502**	40.997**	0.0000003ns	0.0012 ns
Ecotype in decay	2	0.000006 ns	0.00007 ns	0.000021 ns	0.000014 ns	0.3208 ns	0.000019ns	0.0206 ns
Irrigation in ecotype in foliar spraying	4	0.000008 ns	0.00008 ns	0.000004 ns	0.000039 ns	0.6451 ns	0.0000076ns	0.0110 ns
Error	30	0.0000923	0.0011	0.000017	0.0008	2.346	0.0000019	0.0142
Coefficient of variation (%)	-	7.13	8.16	5.96	10.18	4.17	7.21	11.3

ns, ** and *: non significant and significant at the 1% and 5% probability levels, respectively

Table 3- Mean comparison the effect of drought stress on measured traits of Henna

Drought stress	Sweat rate(mmol/m/s)	Proline (mg/ g FW)	Protein (mg/ g FW)
100	7.38a	2.268c	70.10a
75	6.18b	4.176b	64.83a
50	3.66c	6.794a	48.69b

Table 4- Mean comparison the effect of ecotype on measured traits of Henna

Ecotype	Product growth rate (g/m/s /day)	Sweat rate (mmol/m/s)
Shahdad	9.60a	6.21a
Rudbar-e-Jonub	9.02b	5.60b
Bam	9.77a	5.41b

Table 5- Mean comparison the effect of foliar application on measured traits of Henna

Foliar spraying	Leaf Yield Index	Transpiration Rate (mmol/m/s)	Stomatal Conductance (mmol/m/s)	Proline (mg/ g FW)	Leaf Dry Yield (g/m^2)
control	1.94b	5.44b	186b	4.733a	366.08b
Sodium Nitroprusside	2.11a	6.04a	205a	4.09b	402a

Table 6- Mean comparison the interaction of drought stress and ecotype on measured traits in Henna

Test Treatments		Leaf Yield Index	Pure photosynthesis (μmol/m/s)	Stomatal Conductance (mmol/m/s)	Leaf Dry Yield (g/m^2)
100 % F.C.	Bam	2.77ab	26.15a	249a	464.01b
	Rudbar-e-Jonub	2.72ab	26.62a	244a	505.25ab
	Shahdad	3.02a	28.69a	246a	539a
75 % F.C.	Bam	2.28b	22.70b	207b	405.15c
	Rudbar-e-Jonub	1.63c	22.19b	181bc	333.46d
	Shahdad	1.79c	24.02b	203b	351.51d
50 % F.C.	Bam	1.28d	15.16c	140cd	274.53e
	Rudbar-e-Jonub	1.29d	14.58c	131d	290.68e
	Shahdad	1.45cd	17.59c	156c	292.76e

Table 7- Mean comparison the interaction of drought stress and foliar application on measured traits in Henna

Test Treatments		Product growth rate (g/m2/day)	Pure photosynthesis (μmol/m/s)	Chlorophyll a (mg/ g FW)	Chlorophyll b (mg/ g FW)	Chlorophyll Total (mg/ g FW)	Carotenoid(mg/ g FW)	Catalase(unit/ mg protein)
100 % F.C.	control	11.06b	26.42a	0.339a	0.174a	0.532a	0.090a	0.155e
	Sodium nitroprusside	11.91a	27.88a	0.343a	0.178a	0.542a	0.091a	0.152e
75 % F.C.	control	8.30d	21.11b	0.244c	0.126c	0.383c	0.064c	0.292c
	Sodium nitroprusside	9.77c	24.83ab	0.313b	0.162b	0.493b	0.084b	0.249d
50 % F.C.	control	7.57e	15.17c	0.123e	0.064e	0.193e	0.032e	0.468a
	Sodium nitroprusside	8.17d	16.38c	0.198d	0.102d	0.312d	0.053d	0.351b

4.Conclusion

The results of this study showed that although reducing the irrigation water level decreased the photosynthetic pigments, growth indices, net photosynthesis, and ultimately leaf dry matter yield. Sodium nitroprusside foliar spray treatment at 75% and 50% of the agricultural capacity levels resulted in a significant increase in leaf area index, crop growth rate, net photosynthesis, and dry matter yield compared to the control treatment. Furthermore, among the studied ecotypes, the Shahdad ecotype had a more desirable overall performance compared to the Rudbar-e Janubi and Bam ecotypes in terms of the measured traits.

The findings of this study have important implications for the agricultural industry, particularly in arid and semi-arid regions where water scarcity is a major constraint to crop production. Henna is a valuable medicinal and cosmetic plant, and its cultivation is economically significant in many countries. However, its productivity is severely affected by drought stress. The use of SNP as a foliar spray can offer a sustainable and cost-effective approach to enhance the drought tolerance of henna plants, thereby ensuring their productivity and economic viability in water-limited environments.

Future research should focus on field trials and long-term effects to validate the effectiveness of SNP foliar application and explore its potential application in other crop plants.

Ethical Notes

Contributors

The contribution of the authors in the article extracted from the thesis should be as follows:

First author: Data collection, analysis and interpretation of information and results, drafting the article.

Second author: Thesis advisor, research design, supervision of research stages, review and control of results, editing, reviewing, and finalizing the article.

Third author: Thesis consultant, contribution to research design, research supervision, reading and reviewing the article.

Conflict of Interest

According to the authors' statement, there is no conflict of interest in this article.

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