



# The effects of *Marrubium alysson* and *Torilis arvensis* natural and nano extracts on priming of wheat seeds in response to drought

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## Abstract

Agriculture and climate change are inextricably linked in various aspects. Droughts have become more frequent as a result of climate change, having a significant impact on crop productivity. As a result, the current study investigated the effect of seed priming with natural plant extract and biosynthesized nano plant extract as an environmentally friendly tool for mitigating the drought effect on wheat as an economic crop. The study investigates the biosynthesis of Ag-nano particles from extracts of *Marrubium alysson* and *Torilis arvensis*. The UV–Vis spectrophotometer was used to characterize the biosynthesized AgNPs. Wheat grains were primed with *Marrubium alysson* and *Torilis arvensis*, along with their nanoextracts, and grown in different water regimes (100%, 60% and 40% field capacity), as well as hydropriming. Leaves were collected to determine the photosynthetic pigments, phenolics, flavonoids, CAT, GPX, H<sub>2</sub>O<sub>2</sub>, MDA, soluble sugars, and soluble proteins. In comparison with hydropriming seeds, the study discovered that natural and nano extracts significantly increased the CAT and GPX, as well as soluble proteins. Phenolics, flavonoids, soluble sugars, H<sub>2</sub>O<sub>2</sub>, and MDA content all decreased significantly, but pigment content remained unchanged. The study believed that priming wheat with natural and nano extracts, improved drought tolerance through the use of their metabolites, which included soluble sugars, phenolics, and flavonoids, accumulating in other metabolites like lignin, starch, and flavolignan to increase plant tolerance and reduce oxidative damage. Furthermore, nano extracts of *Torilis arvensis* and *Marrubium alysson* may be more effective than plant extracts since they separate from each other in PCA analysis.

**Keywords** Drought · Green synthesis · *Marrubium alysson* · Priming · *Torilis arvensis* · Wheat

## Introduction

Globally changing environmental circumstances have resulted in extensive land aridification, which has had a considerable impact on crop growth (Araus 2002). According to the Intergovernmental Panel on Climate Change (IPCC), the global temperature will rise by an average of 1.1–6.4 °C by the end of the twenty-first century. By reducing natural carbon sources, this dramatically reduces the carbon content

of green plants. Due to increased evaporation, drought areas may rise by 1 to 3% by 2100 (Marček et al. 2019; Sallam et al. 2019). Drought is a major abiotic stressor that contributes to food scarcity. It influences plant development and productivity, limiting food production around the world, particularly in drought-prone areas (Hasan et al. 2021). Plant morphological, physiological, and biochemical systems are adversely affected by drought stress (McKay et al. 2003). It results in a decrease in photosynthesis, stomatal closure, leaf area, and shoot growth, causes problems with the metabolism of carbon and nitrogen (Hussain et al. 2020). Additionally, it disrupts the electron transport chain, which raises the generation of excess reactive oxygen species (ROS) and damages membrane structures by oxidizing membrane lipids (Wang et al. 2018; Li et al. 2019).

Among environmental stresses, drought is one of the major constraining variables for wheat efficiency, where the majority of wheat-producing nations (*Triticum aestivum* L.) frequently endure protracted droughts, especially during

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critical stages of development (El Sabagh et al. 2019; Hus-sain et al. 2019). Wheat is one of the most important cere-als and is the staple food for more than 50% of the world's population (FAO 2014). The wheat area is 219.52 million hectares, and the world agricultural production is 733.91 million tons (World Agriculture Production (2018). It is predicted that wheat production could decline by more than 50% over the next 20 years if current climate change contin-ues (Ahmad et al. 2021; Dutta et al. 2021). Wheat (*Triticum aestivum*) is a large annual shrub that belongs to the Poaceae family (Poaceae) and is commonly used as food. It is an economical, valuable and strategic grain in Egypt, and it is the third highest-yielding grain in the world after maize and rice (Singh et al. 2015). Its production is directly related to food security issues, as it is used as a staple grain in urban and rural communities and as a source of straw for animal feed (Jing et al. 2020).

Divergent strategies are being used to overcome the problems of poor germination and emergence for under prevailing soil water deficit environment. Seed priming in various ways is widely used for improved crop stand establishment. Hydro-priming, chemo-priming, and halo-priming are examples of priming techniques (Carrillo-Reche et al. 2018). In this method, seeds are soaked in water (hydropriming) and aerated solutions (osmo-priming) for a set period to stimulate metabolic processes, which are generally activated during the early stages of germination (Sharma et al. 2019). Various priming solutions, such as nutrients, hormones and organic salts, assist plants in mitigating the negative consequences of abiotic stressors, such as drought stress (Noreen et al. 2021). Furthermore, various nanoparticles have been employed to lessen the negative effects of stress, including gold, silver, iron, copper and zinc oxide, and zinc nanotubes (Yao and Tang 2022). Silver nanoparticles (AgNPs) have strong signaling ability in crops under drought/abiotic stress and provide a favorable moisture gradient in leaves (Taran et al. 2017; Naqvi et al. 2022). Seed priming has gained popularity due to its improved economic outcomes. However, most seed priming techniques involve the use of non-eco-friendly chemicals (Ali et al. 2020). Recently, in parallel with the exogenous use of eco-friendly synthetic organic compounds for the induction of stress tolerance in crops, plant-based extracts have been used as a rich source of organic compounds and termed them as biostimulants (Colla and Roup-hael 2015). Seed priming with specific phytochemicals may be a cost-effective and environmentally friendly alternative to such chemicals (Prabha et al. 2016). During soaking, seeds bind water and absorb protective and biologically active compounds. As a result, the procedure activates various enzymes, metabolic pathways (Forti et al. 2020).

*Marrubium alysson* L (family Lamiaceae) is a dicotyledonous genus of Old-World aromatic plants; It has

been used in traditional medicine for a variety of purposes. It is widely distributed in Egypt along the Mediterranean coast (Popoola et al. 2013). This plant has been the subject of a chemical study leading to the isolation and identification of several diterpenes, sterols, flavonoids and phenolic compounds (Mohamed 2010). The potential of flavonoids and phenolic compounds as antioxidants has been extensively studied (Pourreza et al. 2013). It can be used medicinally to treat cough, colds, and asthma (Louhaichi et al. 2011); it can also be used as antiviral (Edziri et al. 2011). As a result, its biological impact was studied, and strong antioxidant and anti-inflammatory properties were found, and it is now thought to be a successful tactic to mitigate the toxicity caused by anti-cancer medications (Eltamany et al. 2021; Essawy et al. 2014). While as a member of the Apiaceae family, *Torilis arvensis* (Huds.), has been used for hundreds of years as a natural remedy to treat a variety of illnesses, including viruses, fungi, and bacteria (Kalsoom et al. 2019; Zengin et al. 2020). Fruit essential oils include monoterpenes (4.71%), phenols (6.1%), oxygenated sesquiterpenes (64.22%), and sesquiterpene hydrocarbons (19.92%). It was recently classified as one species belonging to the family Apiaceae, which contains a high concentration of flavonoids (Youssef et al. 2023).

Even though Egypt produces a lot of wheat (TOP-15), only 40–45% of the country's needs are met by it. Thus, the purpose of this study is to investigate whether priming wheat with extracts from *Marrubium alysson* and *Torilis arvensis*, as well as their green nano extract, can help alleviate the effects of drought.

## Materials and methods

### Preparation of plant extracts, biosynthesis and characterization of AgNPs

#### Preparation of plant extracts, biosynthesis of AgNPs

The leaves of *Marrubium alysson* and *Torilis arvensis* were collected from Borg El-Arab El-Kadeem and El Maamora, respectively, in Alexandria, Egypt. The leaves were dried in oven at 60 °C until the constant weight is obtained. Then water extract solution was prepared as 2 gm in 100 ml water. For preparation of AgNPs, 10 mL of the prepared extract was typically added to 20 mL of 3 mM aqueous silver nitrate (AgNO<sub>3</sub>) solution (1:2 ratio) and boiling until the reddish brown color obtained, it take for 2 and one hour in case of *Marrubium alysson* and *Torilis arvensis* extract, respectively. The solution incubated at room temperature for 24 h in the dark until the brownish color was developed which indicated the formation of AgNPs (Parashar et al. 2009).

## characterization of AgNPs

The reduction in metal ions was roughly monitored by visual inspection of the solution by the conversion of the pale yellow reaction mixture to a colored solution (Fang et al. 2005). The reduction in pure Ag<sup>+</sup> ions in AgNO<sub>3</sub> into AgNPs was monitored periodically by UV–vis spectroscopy (T80 UV–vis spectrophotometer– double beam) after the dilution of the samples with deionized water (Raut et al. 2009). A UV– vis spectrograph of the AgNPs was recorded by using a quartz cuvette with water as reference. The UV–vis spectrometric readings were recorded at 200–800 nm (Leela and Vivekanandan 2008).

## Seed priming and germination

Seeds of wheat divided into five groups first one primed with water as control, the second primed with *Marrubium alysson* extract, the third with Ag nano *Marrubium alysson* extract, the fourth primed with *Torilis arvensis* extract and the fifth one primed with Ag nano *Torilis arvensis* extract. All groups primed for 12 h. The seeds were transferred to plastic pots filled with a fixed amount of previously acid-washed quartz sand and sowed at 8 cm depth. Pots were placed under natural environmental conditions and were irrigated with water for 21 days using three irrigation patterns (100, 60 and 40% field capacity, filed capacities were maintained by weighting each time). The leaves of seedlings were collected and prepared for physiological analysis (2 replicates).

## Photosynthetic Pigments

According to Metzner et al. (1965), photosynthetic pigments: chlorophyll a, chlorophyll b, and carotenoids, were measured in samples of wheat fresh leaves tissues using acetone 85% and measured as detailed below. The fresh weight (0.1 g) of wheat leaves was grounded in the solvent. The homogenized samples were centrifuged at 3000 rpm, and the supernatant was up to 10 mL with acetone (85%). The absorbance was recorded at 663, 644, and 452 nm by a spectrophotometer (VEB Carl Zeiss) using acetone as a blank. The concentration of the pigment fractions (chlorophyll a, chlorophyll b, and carotenoids) using the following equations:

$$\text{Chl.a} = 10.3 \text{ E}_{665} - 0.918 \text{ E}_{647}$$

$$\text{Chl.b} = 19.7 \text{ E}_{647} - 3.87 \text{ E}_{665}$$

$$\text{Cart.} = 4.2 \text{ E}_{453} - (0.0264 \text{ Chl.a} + 0.426 \text{ Chl.b})$$

The concentrations of chlorophylls and carotenoids were expressed as mg g<sup>-1</sup> fresh weight (FW) of plant material. The letter E refers to the absorbance.

## Estimation of protein content

Protein content was estimated according to the method described by Hartree (1972). One ml of the dry leaf water extract was mixed in a tube with 0.9 reagent A (Two g potassium sodium acetate tartarate + 100 g sodium carbonate were dissolved in 1 L of 0.1 N sodium hydroxide), and incubated in a water bath at 50 °C for 10 min. After cooling to room temperature, 0.1 ml of reagent B (two g potassium sodium acetate tartarate + 1 g CuSO<sub>4</sub>·5H<sub>2</sub>O were dissolved in 100 ml of 0.1 N sodium hydroxide) was added, mixed well and the tubes allowed to stand for 10 min. Three ml of reagent C (one volume Folin–Ciocalteu reagent (BDH), diluted to ten volumes with distilled) were rapidly added with mixing using vortex mixer. The tubes were then incubated at 50 °C for 10 min. After cooling, the color was measured at 650 nm and the concentration was read from standard curve made with serum albumin. The insoluble fraction was deduced by subtracting soluble fraction from total protein.

## Estimation of Reducing sugars

Reducing sugars (RS) were determined by the method described by Dubois et al. (1956). To 2 ml of the methanol extract, 1 ml of 5% phenol followed by 5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> were added rapidly. The tubes allowed to stand for 10 min, and the absorbance was recorded at 490 nm.

## Oxidative stress indicators (MDA and H<sub>2</sub>O<sub>2</sub>)

Lipid peroxidation (MDA) contents were assessed following the procedure given by Jambunathan 2010. Fresh leaves (0.5 g) extracted with 0.1% TCA and centrifuge it for 15 min at 10,000×r. The subtraction of absorbance at 532 nm and 600 nm was done for corrections of unspecific turbidity. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) contents were quantified following the protocol described by Velikova and Yordanov 2000. The leaves (0.5 g) was normalized in 1 mL of 0.1% TCA solution and extracted after centrifugation at 8000×r for 15 min. A reaction solution of 1 M potassium iodide (1 mL), 10 mM K-P buffer solution (0.5 mL) and supernatant (0.5 mL) was prepared to observe the absorbance at 390 nm.

## Estimation of antioxidative enzymes

### Preparation of enzyme extract and assay of enzyme activity

Fresh leaf matter (0.5 g) were ground to a fine powder in liquid N<sub>2</sub> and then homogenized in 5 mL of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM D-isoascorbic acid, 2% (w/v) poly vinyl pyrrolidone (PVP) and 0.05% (w/v) Triton X-100 using a chilled pestle and

mortar. Following the method of Gossett et al. (1994), the homogenate was centrifuged at 10,000  $\times g$  for 10 min at 4 °C and the supernatants were collected and used for the assays of catalase and guaiacol peroxidase.

#### Estimation of catalase (EC 1.11.1.6) activity

Catalase (CAT) activity was determined spectrophotometrically by measuring the rate of  $H_2O_2$  disappearance at 240 nm, taking extinction coefficient ( $\Delta\epsilon$ ) as 43.6  $mM^{-1} cm^{-1}$  (Patterson et al. 1984). The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 10.5 mM  $H_2O_2$  (Miyagawa et al. 2000). The reaction was run at 25 °C for 2 min, after adding the enzyme extract, and the initial linear rate of decrease in absorbance at 240 nm was used to calculate the activity.

#### Estimation of guaiacol peroxidase (EC 1.11.1.7) activity

Guaiacol peroxidase (POD) activity was measured spectrophotometrically at 25 °C by following the method of Tatiana et al. (1999). The reaction mixture (2 ml) consisted of 50 mM potassium phosphate (pH 7.0), 2 mM  $H_2O_2$ , and 2.7 mM guaiacol. The reaction was started by the addition of an enzyme extract. The formation of tetraguaiacol was measured at 470 nm ( $\Delta\epsilon = 26.6 mM^{-1} cm^{-1}$ ).

#### Estimation of total phenolic and flavonoids content

##### Total phenolic content

Fresh leaf tissue (50 mg) was homogenized with 80% methanol and centrifuged at 10,000  $\times g$  for 10 min. One-hundred microliters of the supernatant were diluted with 2 mL of water and 1 mL of Folin–Ciocalteu's phenol reagent and shaken vigorously. Then 5 mL of 20% sodium carbonate solution was added and the volume was made up to 10 mL with distilled water. The contents were mixed thoroughly, and the absorbance was read at 750 nm (Noreen and Ashraf 2009).

##### Total flavonoids content

The content of flavonoids in the examined methanol plant extracts was determined using the spectrophotometric method (Quettier et al. 2000). The sample contained 1 ml of extract and 1 ml of a 2% aluminum trichloride ( $AlCl_3$ ) solution. The samples were incubated for an hour at room temperature. The absorbance was determined using a spectrophotometer at a maximum  $\lambda$  of 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of Rutin

(with concentrations ranging from 10 to 100  $\mu g ml^{-1}$ ) for the calibration line construction. The content of flavonoids in extracts was expressed in terms of rutin equivalent ( $\mu g$  per Rug fresh weight).

#### Statistical analysis

Data was analyzed by using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). The assumptions of variance analysis were tested (for each 100, 60 and 40% FC separately) by ensuring that the residuals were random and homogenous, with a normal distribution. For treatment showing a main effect, means ( $\pm SE$ ) compared by Duncan test.  $P \leq 0.05$  was considered as significant differences between treatments, according to Sokal and Rohlf's technique (1995).

## Results

### Biosynthesis and characterization of AgNPs

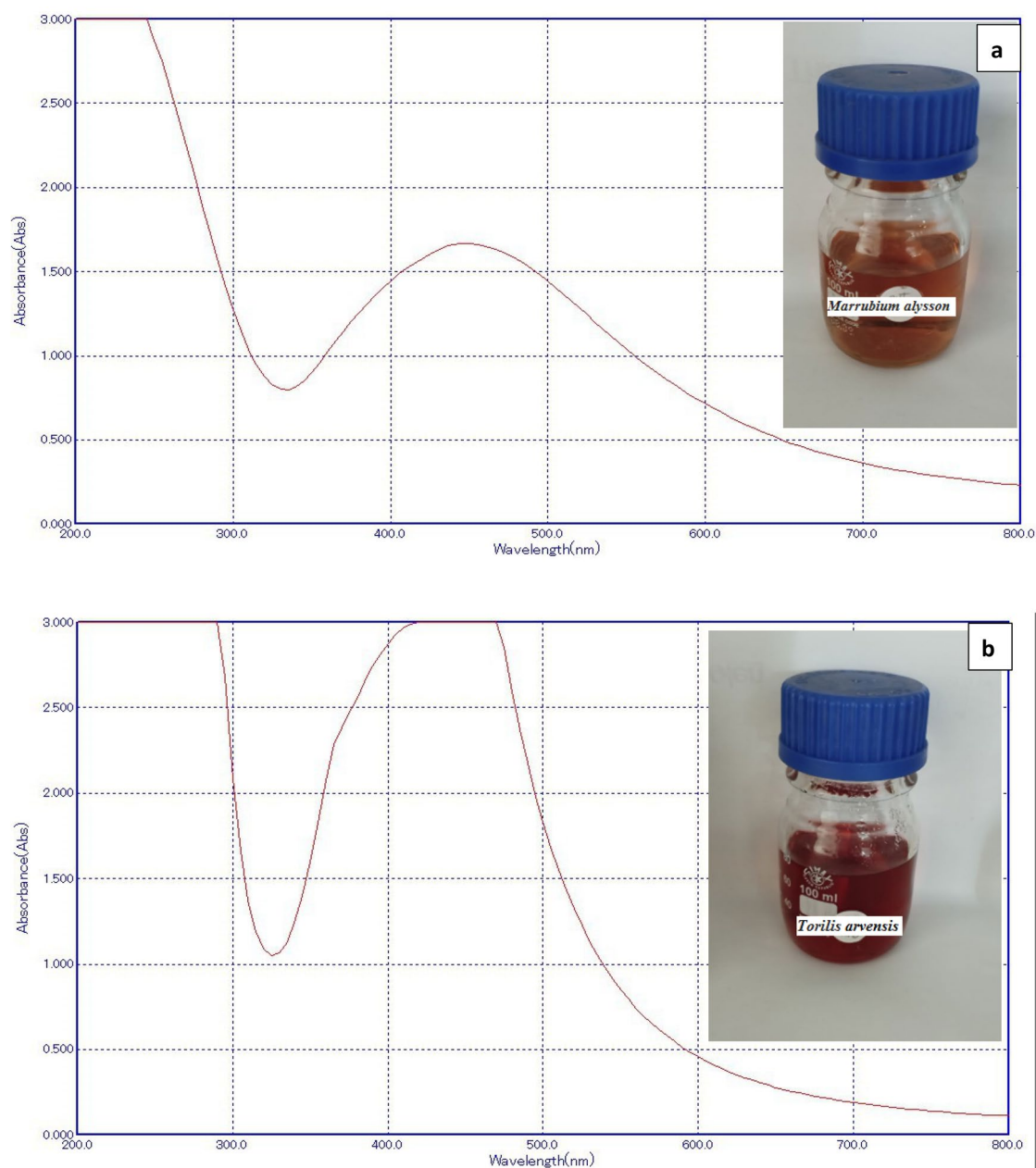
The color of the extracts changed to reddish brown color after addition of  $AgNO_3$  which indicates the reduction in silver ions and the presence of nanoparticles was confirmed by obtaining a spectrum in the visible range of 400–500 nm using UV–visible spectrophotometer (Fig. 1a, b).

### Photosynthetic pigments

Drought stress has no effect on the photosynthetic pigments of wheat and priming with plant extracts or nano extracts has no effect when compared to hydropriming (Fig. 2a–c).

### MDA content and $H_2O_2$ generation

MDA and  $H_2O_2$  content were determined as indicators of oxidative damage in this study. In response to drought stress, MDA and  $H_2O_2$  levels in the hydropriming wheat decrease. In terms of MDA content, priming with plant extracts or nano extracts reduced MDA content in all groups when compared to hydropriming. The maximum decrease in 100% FC in both *Torilis arvensis* and *Torilis arvensis* nano extracts was 65%. *Torilis arvensis* nano extract and *Marrubium alysson* extract produced the greatest reductions in 60 and 40% FC groups, respectively. Priming with plant extracts or nano extracts reduces  $H_2O_2$  content in all irrigation patterns when compared to hydropriming, as shown in Fig. 3B. In 100% FC, the maximum decrease was approximately 62% in both *Marrubium alysson* extract and nano extract. Furthermore, *Marrubium alysson* nano extract demonstrated the greatest reduction in 40% FC by 64% (Fig. 3a, b).

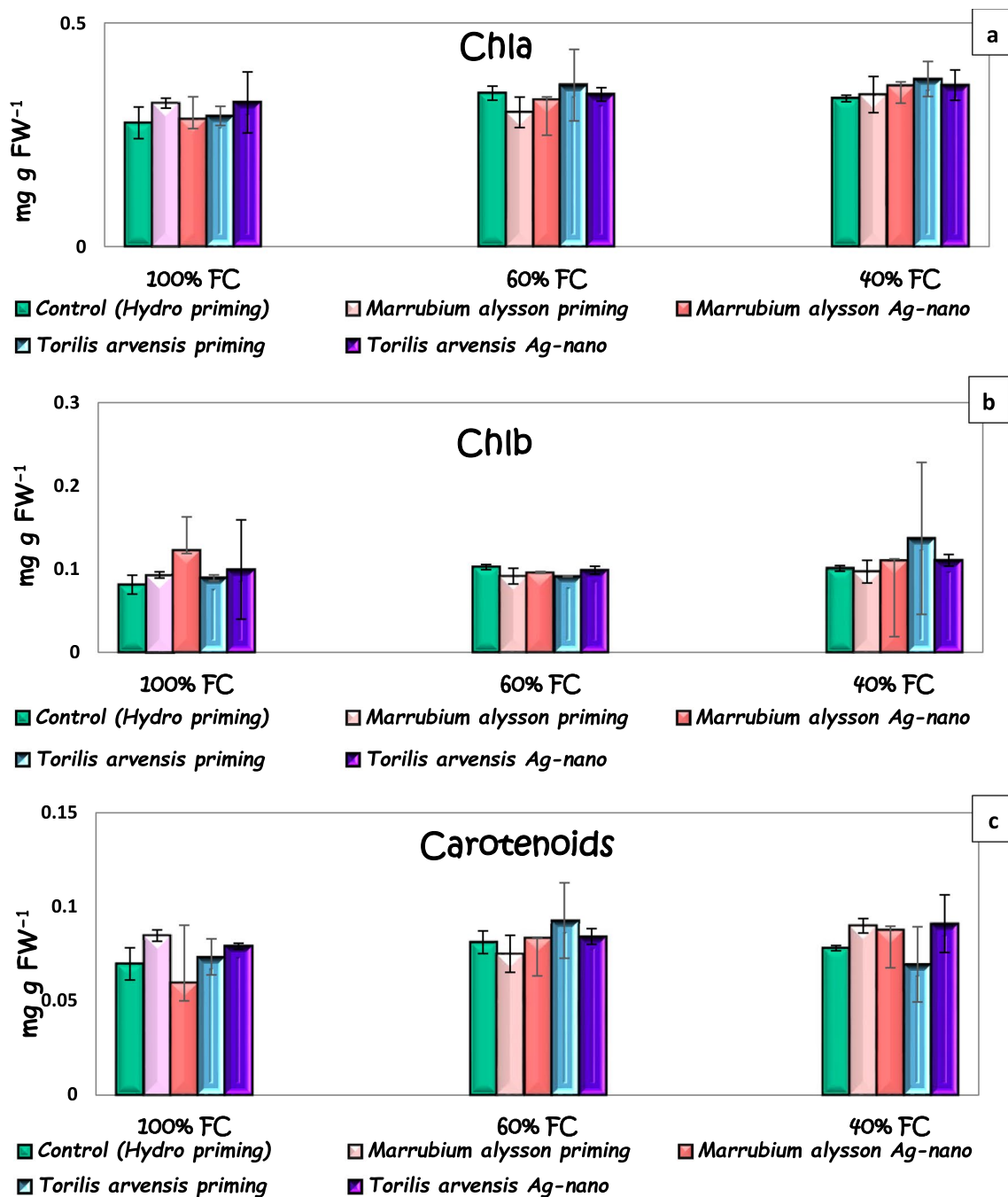


**Fig. 1** UV–vis absorption spectrum of biosynthesized silver nanoparticles using *Marrubium alysson* **a** and *Torilis arvensis* **b** aqueous extract

### Antioxidative enzymes

Catalase and guaiacol peroxidase enzyme activities were measured in wheat in response to different levels of drought and priming. In terms of catalase enzyme activity, the hydropriming wheat shows a decrease in response to drought. However, priming with both plant and plant nano extracts increases catalase activity when compared to hydropriming. We observed that *Marrubium alysson* nano extract increased catalase activity by 8% and 48% in

groups of 100% and 60% FC, respectively. *Torilis arvensis* nano extract priming resulted in a twofold increase in 40% FC (Fig. 4a). On the other hand, in hydropriming wheat, guaiacol peroxidase enzyme activity increased in response to drought stress. In the 100% FC and 40% FC groups, priming with *Torilis arvensis* nano extract increased the GPX activity by two and 1.75 times, respectively. However, in the 60% FC group, the maximum increase was 1.35 times due to *Marrubium alysson* nano extract priming (Fig. 4b).



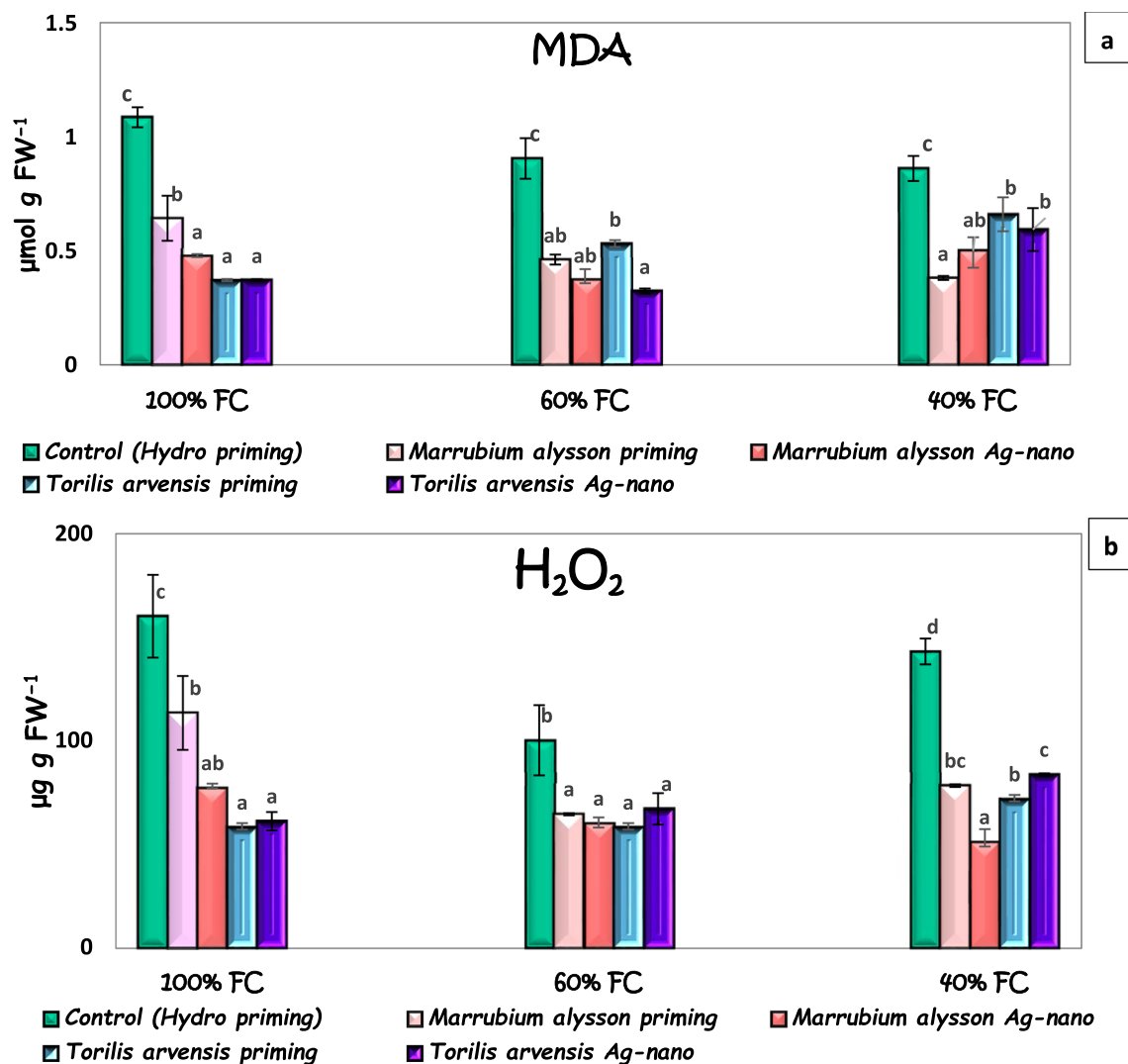
**Fig. 2** The effect of *Marrubium alysson*, *Marrubium alysson* Ag-nano, *Torilis arvensis*, and *Torilis arvensis* Ag-nano extracts on photosynthetic pigments **a** Chl. a content **b** Chl. b content. **c** Carotene content, in wheat plant under drought stress. The values reported in

the figure are means ( $n=2$ )  $\pm$  standard deviation. There was no significant difference as evaluated by Duncan's New Multiple Range Test for each irrigation pattern (100, 60, and 40% FC) separately

### Non enzymatic antioxidant phenolics and flavonoids content

The total phenolic compound in wheat does not change in response to drought stress (Fig. 5a) but priming with plant and nano extracts decreases the phenolic content in all groups

when compared to hydropriming. We discovered that priming with *Marrubium alysson* nano and *Torilis arvensis* extracts reduced it by approximately 40% in a 100% FC group. While priming with nano extracts of *Marrubium alysson* and *Torilis arvensis* reduces by 28% in a group of 60% FC. *Torilis arvensis* extract priming resulted in a maximum decrease of 6%



**Fig. 3** The effect of *Marrubium alysson*, *Marrubium alysson* Ag-nano, *Torilis arvensis*, and *Torilis arvensis* Ag-nano extracts on oxidative damage **a**: MDA content **b**: H<sub>2</sub>O<sub>2</sub> content, in wheat plant under drought stress. The values reported in the figure are means

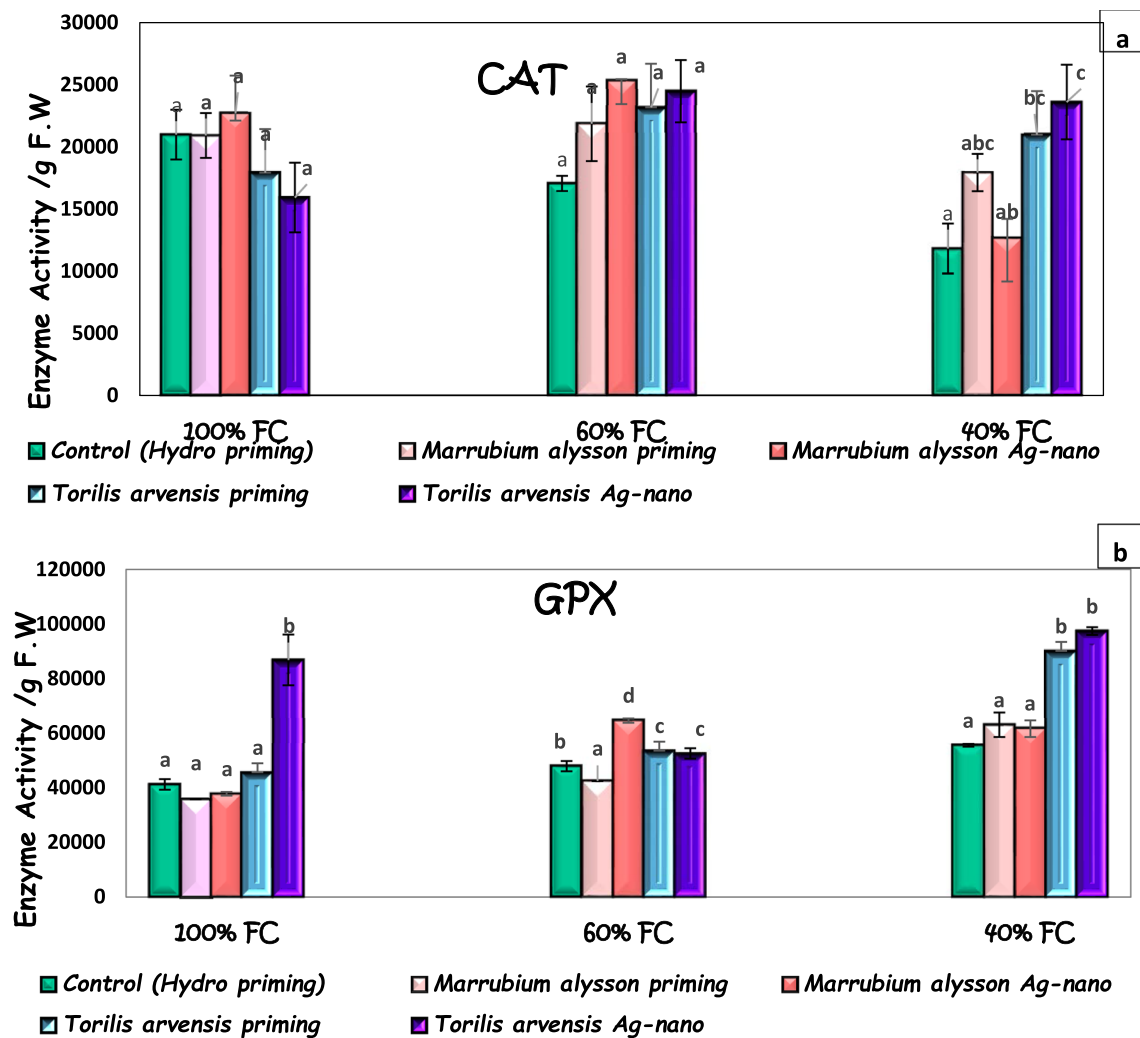
( $n=2$ )  $\pm$  standard deviation. Different letters on the bars are significantly different as evaluated by Duncan's New Multiple Range Test for each irrigation pattern (100, 60, and 40% FC) separately

in the 40% FC group. In terms of flavonoids content, wheat showed a decrease in response to drought stress (Fig. 5b). Furthermore, priming with plant and plant nano extracts reduces flavonoids content in all groups when compared to hydropriming. We observed that *Marrubium alysson* nano extract reduces flavonoids content by 75% and 50% in groups of 100% and 60% FC, respectively. Furthermore, both *Torilis arvensis* and *Torilis arvensis* nano extract priming resulted in a 40% decrease in the 40% FC group.

### Osmo-protectants (soluble sugars and soluble proteins)

Soluble sugars in wheat exposed to 60% and 40% FC decreased to 46% and 50%, respectively. In relation to

hydropriming, we discovered that both plant and nano extracts lower the S.C content during the priming procedure. The extracts of *Marrubium alysson* and nano *Torilis arvensis* showed a maximum decrease of 70% in the control group, as shown in Fig. 6a. While, the extracts of *Marrubium alysson* and nano *Torilis arvensis* showed maximum decreases of 29% and 41% in the 60% and 40% FC, respectively. Conversely, we discovered that drought exposure enhanced the amount of soluble proteins in wheat (Fig. 6b). We discovered that in control gp (100% FC), the maximum increase was 2 and 4.4 folds, respectively, in priming with nano *Marrubium alysson* and nano *Torilis arvensis*. Moreover, in 60% FC gp, nano extracts of *Marrubium alysson* and *Torilis arvensis* demonstrated increases of 1.87 and 2.8 folds, respectively. However, the maximum increase caused by *Marrubium*



**Fig. 4** The effect of *Marrubium alysson*, *Marrubium alysson* Ag-nano, *Torilis arvensis*, and *Torilis arvensis* Ag-nano extracts on antioxidant enzymes activity **a**: CAT **b**: GPX, in wheat plant under drought stress. The values reported in the figure are means

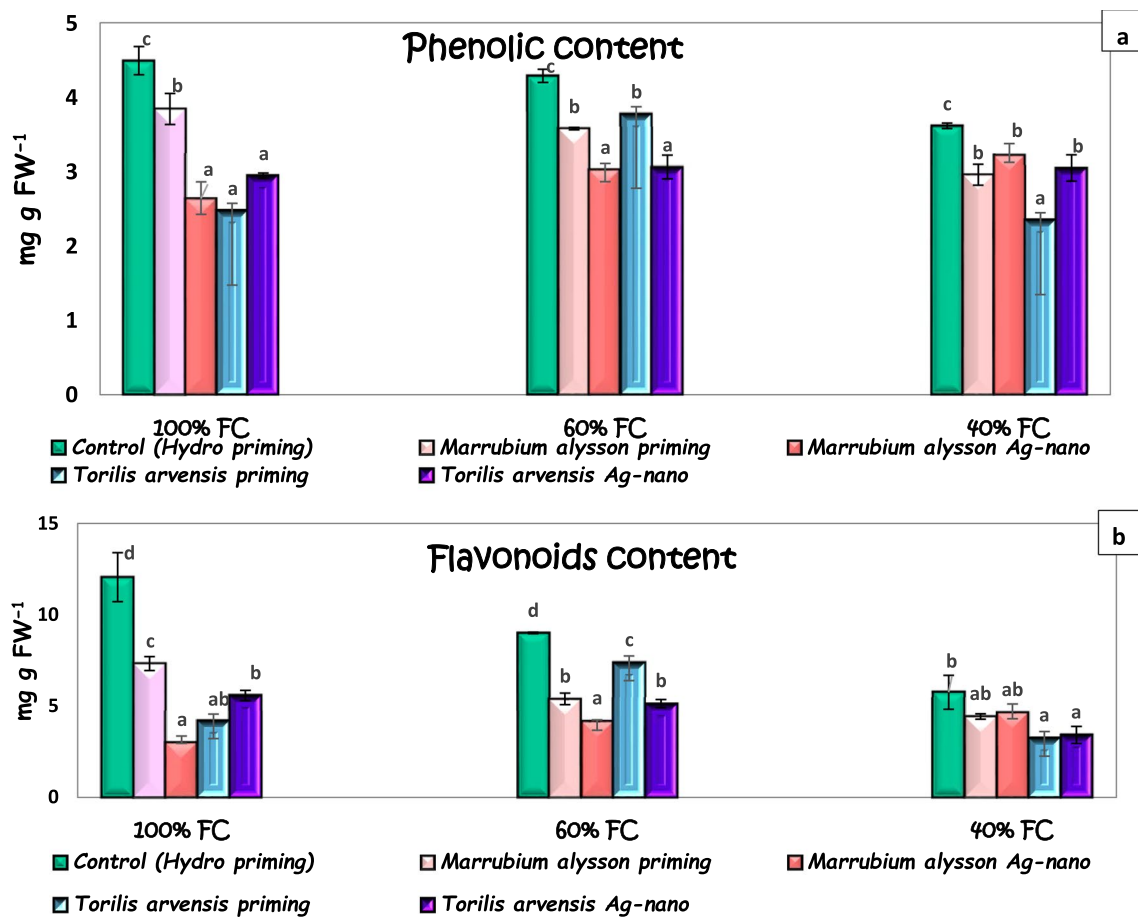
( $n=2$ )  $\pm$  standard deviation. Different letters on the bars are significantly different as evaluated by Duncan's New Multiple Range Test for each irrigation pattern (100, 60, and 40% FC) separately

*alysson* and nano *Marrubium alysson* extracts in 40% FC was 2.6 fold.

### Analysis of principal components

The wheat plants were analyzed using 11 variables, including pigment content (ChlA, ChlB, and carotenoids); phenolic and flavonoids content; osmoprotectant (soluble sugar and soluble proteins); H<sub>2</sub>O<sub>2</sub> and MDA. The first two components, PCA1 and PCA2, represented 44.32% and 18.12% of the total variation, respectively, accounting for 62.44% of the data difference. The PCA2 clearly distinguished two clusters of wheat plants. Cluster 1 was divided into two groups: group 1 contained 100% FC wheat plant control (hydropriming); group 2 contained

60 and 40% FC wheat plants, as well as 100 and 60% FC wheat plants treated with *Marrubium alysson*. The second cluster also included two groups: group 1: all wheat plants primed with *T. arvensis* nano extract; wheat plants primed with *Marrubium alysson* nano extract (40 and 60% FC) and wheat plants primed with *Marrubium alysson* and *T. arvensis* (40 and 60% FC), respectively. Group 2 contains 100 and 40%FC wheat plants treated with *Torilis arvensis*, as well as 100%FC wheat plants treated with *Marrubium alysson* nano extract (Fig. 7a). Concerning the variable plot shown in Fig. 7b, it is clear that phenolic, flavonoids, and soluble sugars are related to each other as well as to H<sub>2</sub>O<sub>2</sub> and MDA. These relation also confirmed in the correlation matrix as it shown in Fig. 8.



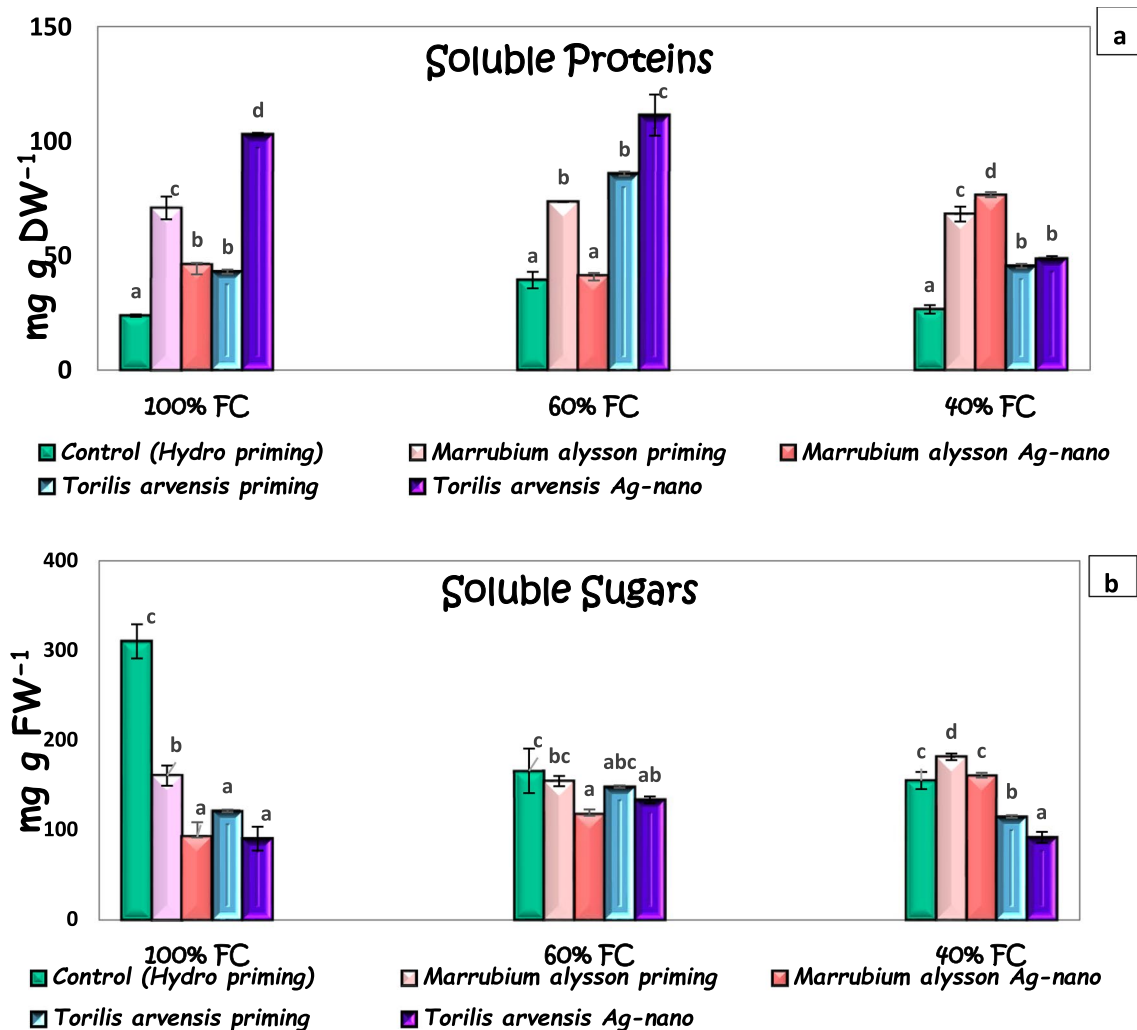
**Fig. 5** The effect of *Marrubium alysson*, *Marrubium alysson* Ag-nano, *Torilis arvensis*, and *Torilis arvensis* Ag-nano extracts on non-enzymatic antioxidant a: phenolic content b: flavonoids content, in wheat plant under drought stress. The values reported in the figure are

means ( $n=2$ )  $\pm$  standard deviation. Different letters on the bars are significantly different as evaluated by Duncan's New Multiple Range Test for each irrigation pattern (100, 60, and 40% FC) separately

## Discussion

Drought is a frequent and recurring natural disaster that affects agriculture and has an impact on society economically. Because the long-term effects of drought are gradual compared to other natural phenomena, they have a greater impact on agricultural production than any other natural phenomenon (Ghaffari et al. 2022). It inhibits the growth of plants by influencing several physiological and biochemical functions, including secondary metabolism, osmotic adjustments, pigment content, membrane integrity, water relations, and producing more reactive oxygen species, such as H<sub>2</sub>O<sub>2</sub> and MDA (Chiappero et al. 2019; Hameed et al. 2011). Since then, the current study aimed to investigate the role of the priming strategy by medicinal plant extracts of *Marrubium alysson* and *Torilis arvensis*, as well as their green nano AgNO<sub>3</sub> extract, in hydropriming; as ecofriendly drought stress remediation (60% and 40% FC), applied on the most economically important wheat crop.

The current study found no significant changes in wheat photosynthetic pigments under drought stress, nor any differences between plant extracts or nano-extracts and water priming. Many species have shown no change or decrease in chlorophyll content during drought stress. Depending on the length and severity of the drought (Kpyoarissis et al. 1995; Sacks et al. 1997). Chlorophyll content in plants can increase or decrease during drought, with the degree of change being closely related to drought tolerance (Yang et al. 2023). Zaefyzadeh et al. 2009 studied 13 durum wheat native varieties from Iran and Azerbaijan and discovered that each wheat variety responded differently to drought stress. Under drought stress, the chlorophyll level of susceptible wheat cultivars decreased significantly, whereas the chlorophyll content of resistant wheat cultivars remained stable. Moreover, damage to chloroplasts by active oxygen species is the primary cause of the reduction in chlorophyll under drought stress (Singh and Thakur 2018). The current study demonstrated a significant reduction in oxidative



**Fig. 6** The effect of *Marrubium alysson*, *Marrubium alysson* Ag-nano, *Torilis arvensis*, and *Torilis arvensis* Ag-nano extracts on osmo-protectants **a**: soluble proteins content **b**: soluble sugars content, in wheat plant under drought stress. The values reported in the

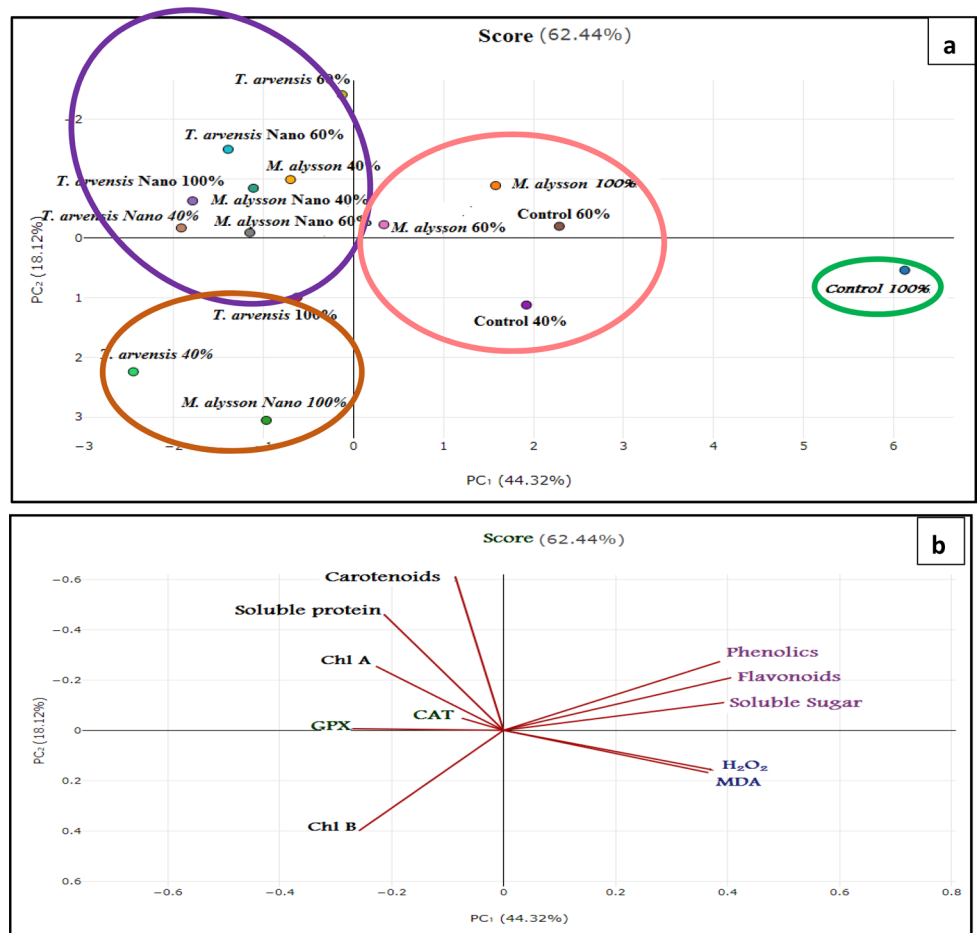
figure are means ( $n=2$ )  $\pm$  standard deviation. Different letters on the bars are significantly different as evaluated by Duncan's New Multiple Range Test for each irrigation pattern (100, 60, and 40% FC) separately

damage, or  $H_2O_2$  and MDA contents, in hydroprimed wheat under drought stress. This attests to the pigment content remaining unchanged. In contrast, our research demonstrates that the green nano extract of *Marrubium alysson* and *Torilis arvensis* lowers the levels of MDA and  $H_2O_2$ . This result is in accordance with that of Alabdallah et al. (2021), who found that green synthesised AgNPs reduce  $H_2O_2$  content in drought-stressed eggplants by upregulating antioxidant enzymes when applied exogenously.

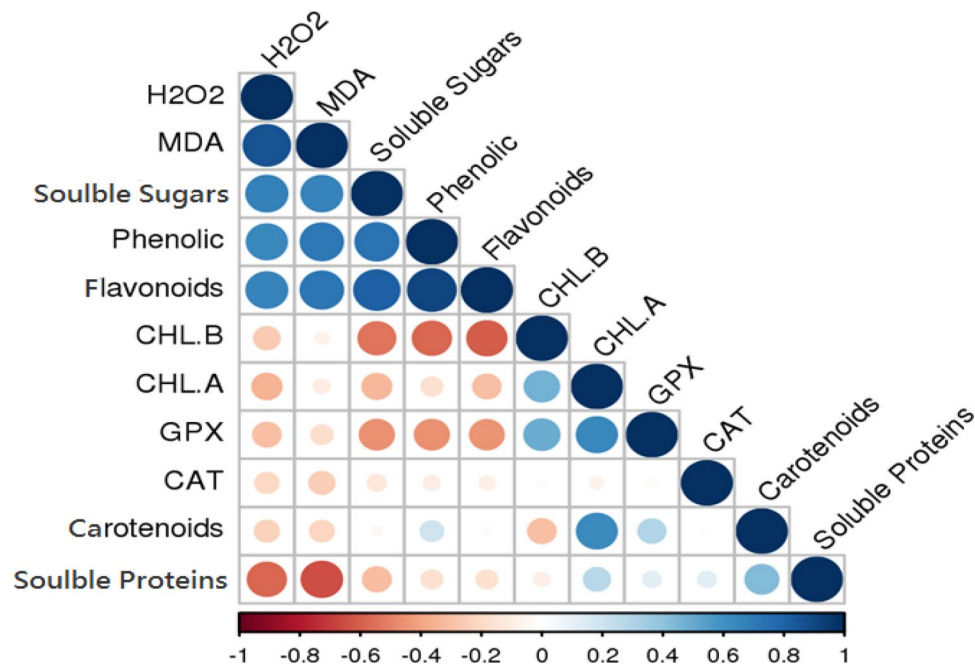
In order to counteract oxidative stress, plants usually possess defence and protection mechanisms called antioxidant systems. These mechanisms include antioxidant enzymes as well as non-enzyme components like phenolic and flavonoid compounds that induce oxidative stress (Ahanger et al. 2017; Chen et al. 2019). In this study, catalase and guaiacol peroxidase were identified as

enzymatic antioxidant mechanisms. Plants that were primed with *Marrubium alysson* and *Torilis arvensis* and their green nano extract showed a significant increase in both CAT and GPX activity. This increase in enzyme activity, combined with lower MDA and  $H_2O_2$  levels, may help to alleviate oxidative damage. These findings are consistent with those of Noman et al (2018), who used sugar beetroot extract as a bio-stimulant to mitigate the negative effects of drought on wheat seed germination and growth. They discovered an increase in the activity of the antioxidant enzymes POD and SOD in primed seeds. Furthermore, Khan et al. 2020 discovered that silver nanoparticles increase CAT and POD activity by 16 and 24% when applied to pearl millet (*Pennisetum glaucum* L.) under different salt stress concentrations, with a noticeable decrease in  $H_2O_2$  and MDA contents. In addition, a recent study by Sarwar

**Fig. 7** Principal component analysis (PCA) of the studied plants, a: Cluster of plants b: Relation of variable based on phytochemical and physiological parameter



**Fig. 8** Person correlation of physiological parameters in the wheat plants used in this study



et al. 2023 found that AgNPs sprayed on *Triticum aestivum* leaves increased SOD, CAT, and POD activity in response to different water levels (100%, 75%, 50%, and 25% FC).

In terms of non-enzymatic components, the study found that drought reduced the content of both phenolic and flavonoids in hydropriming wheat. Plants treated with extract or nano-extract also had lower levels of phenolic and flavonoid content; with a significant correlation with  $H_2O_2$  and MDA (phenolic  $r=0.61$  and  $0.69$ ; flavonoids  $r=0.65$  and  $0.69$  respectively) (Figs. 5, 8) (Fig. 5b). These findings are consistent with Wang et al. 2016, who found a significant decrease in total polyphenols in *Camellia sinensis* (L.) O. Kuntze leaves in response to drought stress, but an increase in flavonoids. The decrease in phenolic and flavonoid content could be attributed to their use in the synthesis of lignin and Flavolignans. Similar to Sassi et al. 2021 investigated the effect of water stress on total phenolic production in four barley cultivars ('Manel', 'Martin', 'Lihane', 'Hope'). They showed that under the influence of water, total phenols accumulate in barley plants and their parts. However, it decreased as water scarcity became more pronounced in the next stage. They explained that as the plant matured, lignin was biosynthesised from free phenols. Plants increase lignin synthesis, a critical component of the cell wall, to prevent water loss during drought (Moura et al. 2010). In addition, Wang et al. 2023 discovered that drought stress caused a significant increase in soluble protein, peroxidase, catalase, and lignin content in flax leaves or roots. Furthermore, Asadi Kavan et al. (2010) demonstrated that exogenous ascorbate could improve *Pimpinella anisum*'s ability to respond to drought stress through various mechanisms, as it reduced flavonoid and anthocyanin contents while keeping MDA content relatively constant. Moreover, ElSayed et al. 2019 and Zangani et al. 2021 found flavonolignan accumulation during drought stress in *Silybum marianum* and treated *Silybum marianum* with sodium nitroprusside respectively.

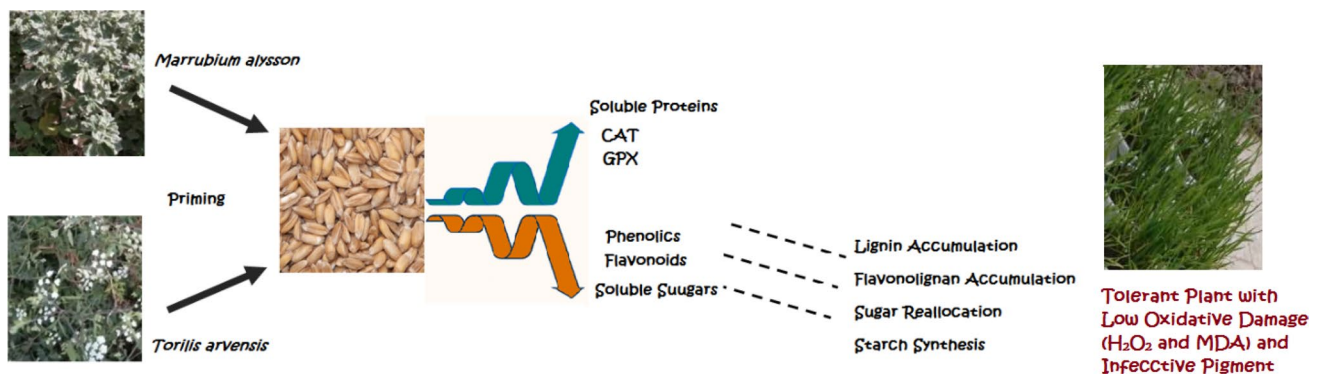
Plants respond to drought by accumulating high intracellular levels of osmoprotectant compounds in order to protect cellular components and restore osmotic balance ((Singh et al. 2015). Soluble protein increased in wheat in response to drought in this study, with a significant increase in treated plants when compared to hydropriming ones (Fig. 6A). This finding is comparable to Ahmadian et al. 2021 study, which found that nano chelated fertilizers increased protein content in wheat under deficit irrigation. Furthermore, Noman et al. 2018 discovered that sugar beetroot extract, when used as a bio-stimulant, increased the total soluble proteins in water stressed wheat. The increase in soluble proteins could be due to de novo amino acid synthesis, which would improve drought tolerance (Yadav et al. 2013). In contrast, drought reduced soluble sugar levels in hydroprimed and treated plants with a significant correlation with  $H_2O_2$  and MDA ( $r=0.63$  and

$0.65$  respectively) (Figs. 6B, 8). Plants regulate metabolic activities related to carbon assimilation during drought stress using various non-structural carbohydrates (Wang et al. 2021). Since the decrease in sugar could be due to reallocation or consumption in starch synthesis. Starch is used to boost antioxidant activity, maintain cell expansion during droughts, and promote carbon uptake by leaves during recovery periods (Abd Elgawad et al. 2020). On the other hand, higher sugar concentrations can cause leaf senescence, indicating that soluble sugar accumulation may have a negative impact on plants (Yuanyuan et al. 2009). Huang et al. (2023) investigated the distribution of non-structural carbohydrates (NSCs) in *Cunninghamia lanceolata* (Lamb.) Hook under drought stress and observed that soluble sugar content decreased while starch content increased in the leaf; they concluded that, in order to ensure osmosis plant allocates more soluble sugars to fine roots while increasing starch content in the leaves. Moreover, O'Brien et al. 2020 examined the relative allocation of NSCs assimilated in seedlings of two tropical tree species and discovered that the drought-tolerant species had lower leaf NSC concentrations but a higher proportion of stem and root. They hypothesized that drought-sensitive species passively accumulated NSCs during water scarcity due to growth inhibition, whereas drought-tolerant species actively responded by allocating NSCs to stem and root organs.

According to PCA analysis (Fig. 7), almost all the nano extract primed plants are distinct from those primed with plant extract, implying that the two nano extracts from *Marrubium alysson* and *Torilis arvensis* have a more potent effect than priming with plant extract. There was no clear distinction between the two plants; however, *Torilis arvensis* might be preferable to *Marrubium alysson*. In terms of physiological parameters, the key physiological metabolites involved in drought tolerance were phenolic, flavonoid, soluble sugar content in reducing the oxidative damage ( $H_2O_2$  and MDA).

## Conclusion

Climate change is expected to increase the amount of land exposed to more frequent and severe droughts. The availability of water eventually limits agricultural production potential. Therefore, the purpose of this study is to investigate the effects of priming wheat seeds with extracts from *Marrubium alysson* and *Torilis arvensis* along with their green synthesised Ag nano extract on plant growth in harsh environments. It has been found that applying extracts and nano extracts can effectively increase the drought tolerance of seedlings. The treated plants exhibited a distinct tolerance mechanism; it was noted that they metabolised sugars, phenolics, and flavonoids in a different way, accumulating other



**Fig. 9** The tolerance mechanism of treated plants with *Marrubium alysson* and *Torilis arvensis* extracts and nano-extracts

metabolites like lignin, flavonolignans, and starch that contribute to reducing oxidative damage and strengthening the tolerance mechanism (Figs. 8, 9). There was no difference between *Torilis arvensis* and *Marrubium alysson* according to the study. *Torilis arvensis*, however, might have a better result. This study provides a positive step towards mitigating the effects of drought using environmentally friendly techniques, nonetheless additional physiological and molecular research is still needed.

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**Data availability** Not applicable.

## Declarations

**Conflict of interests** The authors have no relevant financial or non-financial interests to disclose.

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