Research Article

# Foliar spray can improve rapeseed yield components under continuous irrigation<sup>1</sup>

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# **ABSTRACT**

Applying climate-smart agriculture practices, such as foliar spraying with growth stimulants and improving drought tolerance, seems to be a rational solution in drought-prone areas. The present study aimed to evaluate irrigation regimes (I<sub>1</sub>: full-watered; I<sub>2</sub>: rainfed condition; I<sub>3</sub>: interruption of irrigation at the flowering stage; I<sub>4</sub>: interruption of irrigation at the seed setting stage) and spraying (S<sub>1</sub>: foliar spray with distilled water; S<sub>2</sub>: foliar spray with 250 Mm of thiamin; S<sub>3</sub>: foliar spray with 1 Mm of ascorbic acid; S<sub>4</sub>: foliar spray with 100 ppm of silicon dioxide nanoparticles; S<sub>c</sub>: foliar spray with 500 ppm of zinc oxide nanoparticles) on the morphophysiological traits of rapeseed (Brassica napus L.). The accelerated maturity and the lowest seed yield were recorded under I<sub>2</sub> (1,389 kg ha<sup>-1</sup>), which was 38 % lower than the yield obtained under I<sub>1</sub>. The greatest decrease in the evaluated traits, such as chlorophyll (48 %), leaf relative water content (25 %), number of pods per plant (56 %), plant height (29 %) and canopy spread (24 %), was recorded under I<sub>2</sub>. The lowest plant performance was related to I<sub>2</sub> and I<sub>3</sub>, respectively. The foliar treatments did not mitigate the disruptive effects of I, on plant growth; however, under I, the use of silicon nanoparticles, zinc oxide nanoparticles and thiamine increased some seed yield components.

KEYWORDS: Brassica napus L., irrigation regimes, water stress.

## INTRODUCTION

Rapeseed (*Brassica napus* L.) is an annual vegetable oil crop belonging to the Brassicaceae family. The history of its cultivation dates back more than a thousand years ago, and it plays a significant role in oil production and food security (Borges et al. 2023).

Top rapeseed-producing areas include the European Union (23 %), Canada (21 %), China

# **RESUMO**

Pulverização foliar pode melhorar os componentes de rendimento da colza sob irrigação contínua

A aplicação de práticas agrícolas inteligentes em relação ao clima, como pulverização foliar com estimulantes de crescimento e melhoria da tolerância à seca, parece ser uma solução racional em áreas propensas à seca. Objetivou-se avaliar o efeito de regimes de irrigação (I,: rega completa; I,: condição de sequeiro; I,: interrupção da irrigação na floração; I,: interrupção da irrigação no estágio de desenvolvimento das sementes) e pulverização (S<sub>1</sub>: pulverização foliar com água destilada; S<sub>2</sub>: pulverização foliar com 250 Mm de tiamina; S<sub>3</sub>: pulverização foliar com 1 Mm de ácido ascórbico; S<sub>a</sub>: pulverização foliar com 100 ppm de nanopartículas de dióxido de silício; S.: pulverização foliar com 500 ppm de nanopartículas de óxido de zinco) nas características morfofisiológicas da colza (Brassica napus L.). A maturidade acelerada e o menor rendimento de sementes foram registrados sob I<sub>2</sub> (1.389 kg ha<sup>-1</sup>), que foi 38 % menor que o rendimento obtido sob I<sub>1</sub>. A maior diminuição nas características avaliadas, como clorofila (48 %), conteúdo relativo de água nas folhas (25 %), número de vagens por planta (56 %), altura da planta (29 %) e distribuição do dossel (24 %), foi registrada sob I<sub>2</sub>. O menor desempenho da planta foi relacionado a I<sub>2</sub> e I<sub>3</sub>, respectivamente. Os tratamentos foliares não mitigaram os efeitos disruptivos de I, no crescimento da planta; no entanto, sob I, o uso de nanopartículas de silício, nanopartículas de óxido de zinco e tiamina aumentaram alguns componentes do rendimento de sementes.

PALAVRAS-CHAVE: *Brassica napus* L., regimes de irrigação, estresse hídrico.

(18%), India (13%), Australia (6%), Ukraine (5%), Russia (5%), United States (2%), United Kingdom (1%) and Bangladesh (1%) (USDA 2024), and its world production in 2023 reached 88.7 million tons (FAO 2023).

Despite the relatively high adaptability of rapeseed to diverse climatic conditions, its production rate in the semi-arid regions of Iran is only 138,500 tons (FAO 2023). Previous investigations

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indicate that, among the main reasons for the low production of rapeseed in drought-prone areas of the northwest, the insufficiency of technical information, such as lack of agronomic guidelines, in terms of agro-climatic zoning, improper crop management, especially in water consumption, and extensive climate changes stand out (Bodner et al. 2015). In these areas, there is a lack of climate-smart agricultural practices and, most importantly, severe water shortage (Janmohammadi et al. 2024). At the end of the spring season, the decrease in the amount of rainfall and the simultaneous requirement of other crops for irrigation and the interference of the irrigation program can lead to the occurrence of water shortage and a decrease in rapeseed yield.

Due to its unique physiological characteristics and acceptable drought tolerance, under rainfed conditions, rapeseed can produce a reasonable grain yield, when compared to other brassicas (Aboodeh et al. 2024). However, according to the climatic conditions, amount and distribution of rainfall, it may be cultivated as rainfed or irrigated. However, in the central and west Asia and north Africa, due to specific climatic conditions and the reduction of heavy rains after April, as well as the beginning of the spring crop cultivation, most of the available water is allocated by farmers to other profitable spring crops, which will be able to grow only through irrigation, due to cultivation in dry areas without rain. In such crop rotations, due to the lengthening of irrigation intervals caused by the lack of water resources, the key and critical reproductive stages face water shortage stress (Sayfzadeh et al. 2021). The occurrence of water shortage and incidence of soil moisture stress during different developmental stages with dissimilar intensities reduce the crop yield quantity and quality (Huang et al. 2023).

Although some responses of rapeseed to drought stress have been investigated in detail, the impact of water shortage during reproductive growth periods due to the expansion of spring crops and lack of water resources during these sensitive and key stages have not been well studied for rapeseed. These problems have intensified in recent years, due to climate change fluctuations in rainfall patterns and decreases in rainfall. It seems that rapeseed can be considered a climate-smart crop for drought stress with some management and completion of correctional processes. There are various solutions to adapt to climate change and drought stress. Many

of them require promoting sustainable agriculture and rural development in agricultural zones, and some others depend on the improvement of irrigation structures and agricultural management (Rahimi-Moghaddam et al. 2021). Considering the increasing frequency of dry and hot periods and years with less rainfall than the long-term average, it is necessary to apply climate-smart agricultural management, which consists in an integrated management and implementation plan to reduce the adverse effects of climate change, especially water shortage, as well as to help to improve food security under climate change conditions (Yadav et al. 2023).

Agronomic management improvement is among the low-cost and most effective adaptive mechanisms (Tyagi et al. 2020). The foliar spray of some growth regulators, stimulators of defense systems, and some elements in the dimensions of nanoparticles can be used as agronomic interventions for drought management (Hong et al. 2021). A previous investigation showed that thiamine (vitamin B<sub>1</sub>) is considered an essential cofactor for the activity of some key enzymes on the strengthening of plant biofortification and health, thus improving the crop performance (Fitzpatrick & Chapman 2020). The foliar application of thiamine in pea (*Pisum sativum*), for example, increased the root and shoot growth by increasing antioxidant activities and the accumulation of proteins (Kausar et al. 2023).

The foliar spray of silicon nanoparticles in rapeseed under drought stress conditions improved some physiological components such as chlorophyll, photosystem quantum efficiency and leaf relative water content (Sajed Gollojeh et al. 2020). The positive effect of zinc nanoparticles on rapeseed under drought stress has also been reported by Ghassemi Golezani et al. (2023). The exogenous application of zinc nanoparticles in foxtail millet (Setaria italica) increased the water-use efficiency, amplified the resistance to drought and improved the nitrogen content and oil percentage (Kolenčík et al. 2019). Ascorbic acid (vitamin C) also plays an important role in cleaning reactive-oxygen species (Zheng et al. 2024). Vitamin C can be involved in enzymes as cofactors, such as violaxanthin de-epoxidase, which is associated with the xanthophyll cycle and photoprotection (Sharma et al. 2019).

Therefore, it seems that the foliar spraying of the aforementioned compounds under drought stress conditions is promising for improving the rapeseed yield. Thus, this experiment was designed to evaluate the impacts of diverse foliar spraying treatments under irrigation regimes and drought stress conditions during the reproductive stages on agronomic and morphophysiological traits of rapeseed grown in a xerothermic region of Qazvin, in the northwest of Iran.

## MATERIAL AND METHODS

The experiment was carried out at the Qazvin agricultural research station (36°15′N, 50°03′E and altitude of 1,275 m), during the 2022-2023 crop season. The region is located in the northwest of Iran and has a semi-arid climate, where most of the annual rainfalls occur during the cold season and the soils are classified as Lixisols (Azarneshan et al. 2018). The total amount of rainfall during the growing season was 253 mm, and the soil moisture and temperature regimes were dry xeric and mesic, respectively (Azarneshan et al. 2018). Based on the soil analysis and the data available at the station, the soil texture was clay loam up to 30 cm deep and included 22 % of sand, 37 % of silt and 31 % of clay. The soil chemical characteristics were: electrical conductivity: 0.92 ds m<sup>-1</sup>; total N: 0.17 %; K: 293 mg kg<sup>-1</sup>; organic matter: 1.42 g kg<sup>-1</sup>; available P: 19.39 mg kg<sup>-1</sup>; and pH: 7.71. The amount of rainfall and monthly average air temperatures during the rapeseed growing season are shown in Figure 1.

The experiment was conducted as a splitplot randomized complete block design, with three replications.

The main plots included four irrigation regimes:  $I_1$ : watered;  $I_2$ : rainfed condition;  $I_3$ : interruption of

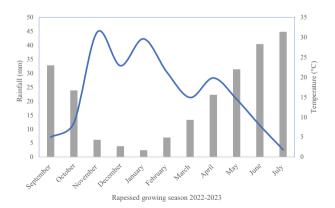


Figure 1. Meteorological data recorded at the Qazvin agricultural research station (Iran), during the 2022-2023 rapeseed growing season. The blue line is the monthly rainfall trend and the columns are the mean monthly temperature.

irrigation at the flowering stage; I4: interruption of irrigation at the seed setting stage. Under I<sub>1</sub>, the plants never faced withering conditions, even temporarily. In this treatment, the plants were irrigated according to climatic conditions such as temperature, rainfall, wind, evaporation and transpiration waste at time intervals of 4-10 days. The volume of water in each irrigation was 30-80 mm, depending on the climatic conditions. The split-plot 4 (irrigation levels)  $\times$  5 (leaf spraying treatments) arrangement was used to assign treatments to the experimental plots. Each experimental plot was 3 × 5 m, and each experimental replication included 20 experimental units. One meter of land was left uncultivated to avoid mixing the effects of irrigation and foliar spraying between adjacent plots. The sub-plots were assigned to foliar spray treatments consisting of: S<sub>1</sub>: foliar spray with distilled water (control); S<sub>2</sub>: spray with 250 Mm of thiamin; S<sub>3</sub>: foliar feeding with 1 Mm of ascorbic acid; S<sub>4</sub>: exogenous application with 100 ppm of silicon dioxide nanoparticles; S<sub>5</sub>: spray with 500 ppm of zinc oxide nanoparticles. The silicon dioxide and zinc oxide nanoparticles were obtained from the Ebtekar Armina Sustainable Engineering Company. According to the images provided by a scanning electron microscope, the dimensions of the particles were smaller than 100 nm. Foliar spraying was replicated four times at the end of the rosette stage (BBCH: 14) and at the middle of the stem elongation (BBCH: 35), flowering (BBCH: 65) and seed setting (BBCH: 75). Tween-20 (0.1 %) was used for a better adhesion of the sprayed material to the rapeseed shoots and leaves. The seeds of the winter rapeseed (*Brassica napus* L.), Talaieyeh variety, were provided by the Seed and Plant Improvement Institute (Karaj, Iran). This winter cultivar is suitable for autumn cultivation in cold and moderately cold regions. The average growth period of this variety until ripening is 260 days. The seed of this free-pollinating rapeseed variety was received four decades ago, under the name of Kabri, from the University of Göttingen, Germany, and was imported by Iran for breeding. However, due to the resemblance of climatic conditions during the last twenty years, these results can be attributed to long-term years.

The field was tilled with moldboard plow in early September 2022, and 10 t ha<sup>-1</sup> of rotted farmyard manure were used. Then, secondary tillage was done with a disc and furrower. The seed sowing was performed manually on September 27, at a depth of 1 cm, on the smoothed peak of

ridges. The rows were planted with 50 cm inter-row spacing. The density for the rapeseed variety used under the mentioned climate conditions is about 50 plants m<sup>-2</sup>, as recommended by local agriculture extension specialists. At the sowing stage, the intrarow distance was considered near to 5 cm. Each experimental plot had 6 planting rows with length of 5 m. Due to the inappropriate distribution of rainfall and the possibility of seed deterioration under rainfed conditions, as well as to ensure the germination and establishment of seedlings, all plots were irrigated immediately after planting. The water requirement of rapeseed in winter crops in cold regions is estimated to be 400-500 mm during the growth period (Eskandari & Kazemi 2024), being 500-700 mm for spring cultivation. For a better establishment of plants and relative uniformity of the growth stages, all the test plots were watered immediately after planting the seeds (100 mm), in order to provide moisture up to the field capacity. According to the climatic and edaphic conditions of the region, the rapeseed water requirement is estimated at 600-800 (Eskandari & Kazemi 2024). Soil water deficit during the planting stage causes a delay in the emergence of seedlings and sometimes failure in cultivation (Channaoui et al. 2019). Due to the low soil moisture in the seed planting stage (18 %), the implement of one irrigation was necessary. Irrigation was carried out with polyethylene pipes and drip tape, through drippers with 3-cm intervals on the irrigation brigade tape. After each irrigation, the soil moisture reached the field capacity (33 %). During the entire growing season, 4,000-5,000 m<sup>3</sup> of water were consumed, according to the plant requirements and irrigation treatments. The irrigation depth was 100 mm; however, the irrigation intervals varied according to the climatic conditions and water requirements during the plant growth stages. To prevent moisture leakage between the main plots, 2 m were considered as a margin. The dose of 120 kg ha<sup>-1</sup> of nitrogen from urea fertilizer was used in three stages: one-third during planting, one-third during stem elongation and the rest during the early flowering stage. A total of 80 kg ha<sup>-1</sup> of phosphate from triple-superphosphate (44 % of P<sub>2</sub>O<sub>5</sub>) and 40 kg ha<sup>-1</sup> of K from potassium sulfate were consumed as band application during the final stage of ridge formation. Under full irrigation and drought stress conditions in the reproductive stage (until the onset of drought stress), irrigations were repeated

at intervals of 4-11 days, according to the depletion of 60 % of soil moisture, which was evaluated by a time-domain reflectometer. The leaf chlorophyll was measured using a SPAD-502plus chlorophyll meter (Konica Minolta-USA) at the beginning of the seed setting. Weed control was done manually several times during the stemming stage.

To determine the leaf relative water content (RWC), in the middle of the seed setting stage, five leaves were randomly picked in each experimental plot, placed in an ice flux, transferred to the laboratory, and had their fresh weight measured (FW). Then, discs with 2 cm of diameter were prepared using a punch machine and placed in distilled water for 12 hours, under the dark and cool temperature of a refrigerator, after that reaching the turgid state and having their weight recorded (TW). After drying the leaves in an oven at a temperature of 70 °C for 24 hours, their dry weight (DW) was reached. The RWC was determined as follows: RWC (%) =  $[(FW - DW)/(TW - DW)] \times$ 100 (Pieczynski et al. 2013). At the full maturity stage, the plants were randomly harvested using a 1-m<sup>2</sup> quadrant. After drying the harvested plants in an oven at a temperature of 70 °C for 48 hours, the aboveground biomass was calculated with a sensitive scale. Subsequently, the number of pods per plant, number of seeds per pod and 1,000-seed weight were evaluated. The harvest index was obtained from the ratio of seed yield to aboveground biomass. For the extraction of vegetable oil, the dried seeds under room temperature were milled and hexane was used as an extraction solvent (AOCS 2004), and the oil percentage was measured by the Soxhlet extractor (Mamnabia et al. 2020).

The analysis of variance for the evaluated traits was performed with the SAS software, whereas the comparison of means was carried out using the LSD test at the level of 5 %, and the principal component analysis using the Minitab software. Figures and box plots were drawn with the Excel and SPSS softwares.

### RESULTS AND DISCUSSION

The investigation of rapeseed phenology showed that the main effects of the irrigation (I) and foliar spraying (S) treatments, as well as the mutual effects of  $I \times S$  at the level of 1 %, had a significant effect on days to maturity (DTM). The highest DTM value was recorded under full-irrigation conditions

with external application of ascorbic acid (286 days), and the shortest growth period was recorded under rain-fed conditions without the foliar spraying of stimulants (233 days). The cessation of irrigation at the flowering stage ( $I_3$ ) accelerated the maturity by about 20 days, when compared to full-irrigated conditions. The extent of the canopy is one of the important vegetative traits and can directly affect the amount of photoassimilates production. A high canopy spread can increase the ability of the source to receive more light and provide more photoassimilates (Yan et al. 2024). The cessation of irrigation at

the seed setting stage accelerated this process by about 8 days (Table 1). A brief comparison of DTM between the levels of foliar sprays showed that the ascorbic acid could significantly increase the length of the development period, if compared to the other treatments.

The mutual I  $\times$  S effects for the canopy spread were significant at the statistical level of 5 %. The widest canopy span (52.44 cm) was recorded under full-irrigation and with the thiamin foliar spray. The plants grown under the condition of irrigation interruption in the seed setting stage (I<sub>s</sub>) and treated

Table 1. Effect of the irrigation interruption in the growth stages and foliar application growth stimulators on the rapeseed (*Brassica napus* L.) morphological traits.

| Treatments   | DTM        | СН       | RWC       | NPP           | TSW      | SY           | HI         |
|--|------------|----------|-----------|---------------|----------|--------------|------------|
| I  | 271.66 a   | 68.46 a  | 79.25 a   | 160.93 a      | 3.42 a   | 2,255.86 a   | 25.85 a    |
| $I_2^{'}$  | 239.92 d   | 36.45 d  | 59.14 d   | 86.79 с       | 2.72 c   | 1,389.07 d   | 23.66 с    |
| $I_3^2$  | 251.92 с   | 48.23 c  | 67.18 c   | 142.71 b      | 2.95 b   | 1,688.29 c   | 23.19 с    |
| $I_4^3$  | 262.46 b   | 52.85 b  | 69.36 b   | 158.28 a      | 2.90 b   | 2,113.00 d   | 25.01 b    |
| $S_0$  | 248.94 d   | 47.68 с  | 65.67 с   | 129.03 с      | 2.91 b   | 1,764.74 b   | 24.11 a    |
| $S_1^0$  | 254.14 с   | 51.33 ab | 68.64 b   | 135.93 b      | 2.92 b   | 1,879.19 a   | 24.71 a    |
| $S_2$  | 263.21 a   | 53.34 a  | 67.50 b   | 144.90 a      | 3.02 a   | 1,875.97 a   | 24.07 a    |
| $egin{smallmatrix} \mathbf{S}_2 \\ \mathbf{S}_3 \end{bmatrix}$ | 256.63 с   | 51.74 ab | 71.03 a   | 137.08 b      | 3.06 a   | 1,900.62 a   | 24.91 a    |
| $S_4^3$  | 259.54 b   | 53.40 a  | 70.72 a   | 135.93 b      | 3.07 a   | 1,886.26 a   | 24.34 a    |
| $I_1S_0$   | 264.66 d   | 63.64 d  | 76.51 c   | 149.33 cd     | 3.42 ab  | 2,116.27 cd  | 25.43 bc   |
| $I_1 S_1$  | 265.00 cd  | 71.64 ab | 75.08 c   | 159.00 bc     | 3.39 b   | 2,252.36 ab  | 25.97 ab   |
| $I_1 S_2$  | 286.66 a   | 68.43 bc | 80.06 b   | 172.00 a      | 3.38 b   | 2,279.26 ab  | 24.99 bcd  |
| $I_1 S_3$  | 271.00 b   | 66.33 cd | 83.20 a   | 158.66 bc     | 3.54 a   | 2,320.42 a   | 27.47 a    |
| $I_1S_4$   | 270.71 b   | 72.28 a  | 81.42 ab  | 165.66 ab     | 3.37 b   | 2,310.9 a    | 25.40 bc   |
| $I_2S_0$   | 233.56 j   | 33.60 k  | 54.83 i   | 83.96 g       | 2.52 h   | 1,321.00 f   | 23.17 def  |
| $I_2S_1$   | 243.56 j   | 35.40 k  | 60.87 h   | 86.46 g       | 2.70 g   | 1,389.67 f   | 23.44 def  |
| $I_2^2 S_2^1$  | 243.36 i   | 41.20 j  | 57.05 i   | 90.42 g       | 2.86 efg | 1,386.67 f   | 23.37 de   |
| $I_2^2 S_3^2$  | 252.18 fgh | 36.40 k  | 61.15 h   | 85.42 g       | 2.76 g   | 1,423.00 f   | 24.06 cde  |
| $I_2S_4$   | 249.57 gh  | 35.69 k  | 61.82 h   | 87.71 g       | 2.86 efg | 1,425.00 f   | 24.30 cde  |
| $I_3^2 S_0^4$  | 243.69 i   | 44.61 ij | 65.13 g   | 136.18 f      | 2.72 g   | 1,628.67 e   | 23.10 ef   |
| $I_3S_1$   | 261.33 de  | 46.34 hi | 66.83 fg  | 144.95 def    | 3.03 cd  | 1,758.41 e   | 24.48 bcde |
| $I_3^3 S_2^1$  | 252.84 fg  | 51.10 fg | 69.13 def | 147.87 de     | 3.04 def | 1,713.60 e   | 23.37 def  |
| $I_3^3 S_3^2$  | 252.18 fgh | 47.29 hi | 66.30 fg  | 145.92 def    | 3.14 c   | 1,662.72 e   | 22.04 f    |
| $I_3S_4$   | 249.57 gh  | 51.82 fg | 68.46 ef  | 138.64 ef     | 2.84 fg  | 1,678.08 e   | 22.16 f    |
| $I_4^3S_0^4$   | 256.66 ef  | 48.86 gh | 66.22 fg  | 146.66 de     | 2.83 fg  | 1,993.00 d   | 24.75 bcde |
| $I_4S_1$   | 270.00 bc  | 52.00 fg | 71.76 d   | 153.33 cd     | 2.87 g   | 2,116.33 cd  | 24.99 bcd  |
| $I_4S_2$   | 256.66 ef  | 52.63 f  | 66.98 fg  | 159.33 abc    | 2.97 g   | 2,128.33 bc  | 24.56 bcde |
| $I_4^4S_3^2$   | 270.00 bc  | 56.93 e  | 70.66 de  | 169.33 ab     | 2.83 g   | 2,196.32 abc | 26.07 ab   |
| $I_4^4S_4^3$   | 270.00 bc  | 53.88 f  | 71.20 de  | 163.66 ab     | 3.01 cd  | 2,196.32 abc | 24.69 bcde |
|  |            |          |           | ficance level |          |              |            |
| I  | 0.0001     | 0.0001   | 0.0001    | 0.0001        | 0.0001   | 0.0001       | 0.0001     |
| S  | 0.0001     | 0.0001   | 0.0001    | 0.0001        | 0.0001   | 0.0009       | 0.2730     |
| $I \times S$   | 0.0001     | 0.0007   | 0.0059    | 0.1960        | 0.0006   | 0.7220       | 0.1920     |
| CV (%)   | 1.18       | 4.15     | 3.69      | 4.33          | 2.94     | 8.23         | 6.53       |

 $I_1$ : full-irrigation;  $I_2$ : rainfed condition;  $I_3$ : interruption of irrigation at the flowering stage;  $I_4$ : interruption of irrigation at the seed setting stage;  $S_0$ : foliar spray with distilled water;  $S_1$ : foliar spray with thiamin (250 ppm);  $S_2$ : foliar spray with ascorbic acid (1 mM);  $S_3$ : foliar spray with silicon dioxide nanoparticles (100 ppm);  $S_4$ : foliar spray with zinc oxide nanoparticles (500 ppm). DTM: days to maturity; CH: chlorophyll content (represented by the measured SPAD value); RWC: leaf relative water content (%); NPP: number of pods per plant; TSW: 1,000-seed weight (g); SY: seed yield (kg ha<sup>-1</sup>); HI: harvest index (%). At the significance level, p-values lower than 0.05 (p < 0.05) and 0.01 (p < 0.01) are significant at 95 and 99 %, respectively. The means with different letters in each trait (column) are statistically different.

with thiamine were in the next position (52.13 cm). However, the plants grown under rainfed conditions ( $I_2$ ) and without the use of growth stimulants, as well as the plants treated with zinc nanoparticles under  $I_2$ , had the lowest canopy width of 34.93 cm and 35.98 cm, respectively (Figure 2).

The chlorophyll content assessment showed that the mutual effects of I × S were significant for this component (Table 1). The foliar spraying of Zn nanoparticles + I<sub>1</sub> resulted in the highest chlorophyll content (72.28), whereas the lowest amount of chlorophyll was recorded under I<sub>4</sub> without the use of growth stimulants or thiamine foliar application, with values of 33.60 and 35.40, respectively. The foliar spraying with zinc nanoparticles and ascorbic acid increased the leaf chlorophyll content by 12 and 11 %, when compared to the control. Our findings confirmed the results by Elshoky et al. (2021) and Kausar et al. (2023), who observed that the foliar spraying of zinc nanoparticles improved the quantum yield of the photosystem II (PSII), the photochemistry of photosystem I (PSI) and the chlorophyll content, and finally the amount of sugar biosynthesis and the ability of the source.

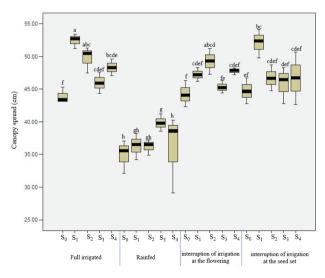


Figure 2. Mean comparison of the canopy spread under irrigation regimes and interruptions at different reproductive stages, along with foliar spraying with growth stimulants on rapeseed. S<sub>0</sub>: foliar spray with distilled water; S<sub>1</sub>: foliar spray with thiamin (250 ppm); S<sub>2</sub>: foliar spray with ascorbic acid (1 mM); S<sub>3</sub>: foliar spray with silicon dioxide nanoparticles (100 ppm); S<sub>4</sub>: foliar spray with zinc oxide nanoparticles (500 ppm). The dashed line in the box indicates the mean of the combined treatment. Boxes with different letters have statistically significant differences at the level of 5 %.

The relative water content (RWC) was strongly affected by the irrigation and foliar spraying treatments. The lowest RWC was recorded under rainfed conditions without the exogenous application of stimulants (54.83 %), and the highest amount was observed under  $I_1$  + silicon and zinc nanoparticles, with values of 83.20 and 81.42 %, respectively (Table 1).  $I_3$  and  $I_4$  reduced the RWC by 15 and 11 %, when compared to full-irrigation.

The above ground biomass (AGB) measurement indicated that the I × S effects were statistically significant at the level of 1 %. Plants grown under full-irrigation with the application of ascorbic acid (9,128 kg ha<sup>-1</sup>) and zinc nanoparticles (9,100 kg ha<sup>-1</sup>) produced the highest AGB. The lowest AGB, regardless of the type of foliar spraying, was recorded under rainfed conditions, with an average of 5,869 kg ha<sup>-1</sup>. The foliar spraying with ascorbic acid and zinc nanoparticles, if compared to the other foliar treatments, produced the highest AGB: 7,757 and 7,727 kg ha<sup>-1</sup>, respectively. The AGB under the mentioned conditions was about 7 % higher than for the plants grown without the foliar spraying of growth stimulants (Figure 3).

The foliar application of zinc oxide nanoparticles increased the vegetative and reproductive growth of wheat under optimal moisture conditions, whereas the foliar spraying with zinc nanoparticles increased the leaf chlorophyll content and aboveground biomass, decreased the oxidative stress, and increased the activity of antioxidant enzymes (Adrees et al. 2021). Their results show that the foliar spraying of growth stimulants, especially useful nanoparticles, can be an efficient method to increase the growth and yield of crops under drought-stress conditions. The use of nanoparticles in soil environments can also improve growth by changing the rhizospheric environment (Zhang et al. 2024). The effectiveness of the foliar sprays was superior under optimal moisture conditions than under stress conditions.

The evaluation of the number of pods per plant (NPP) showed that the plants grown under full-irrigation and  $I_4$  had the highest number of pods. The NPP under rainfed conditions was 44 % lower than for plants grown under full-irrigation. The most positive effect of foliar spraying on NNP was related to the ascorbic acid, which increased this component by 15 %, when compared to the control. The mutual  $I \times S$  effects were significant for number of seeds per pod (NSP), being the highest NSP recorded under  $I_1$ +

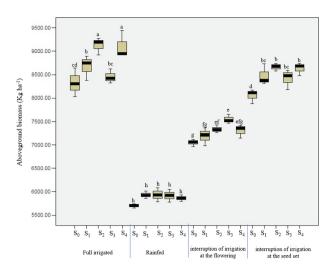


Figure 3. Investigation of the effect of soil moisture treatments and spraying with growth stimulants on aboveground biomass in rapeseed grown in a semi-arid region of Qazvin (Iran). S<sub>0</sub>: foliar spray with distilled water; S<sub>1</sub>: foliar spray with thiamin (250 ppm); S<sub>2</sub>: foliar spray with ascorbic acid (1 mM); S<sub>3</sub>: foliar spray with silicon dioxide nanoparticles (100 ppm); S<sub>4</sub>: foliar spray with zinc oxide nanoparticles (500 ppm).

zinc nanoparticles (22.61) and the lowest one under  $I_2$  + without the foliar spraying of growth stimulants (12.94). Stopping irrigation at the flowering stage reduced the NSP by 18 %, when compared to full-irrigation. However, the NSP of the plants grown under rainfed conditions and irrigation interruption at the seed setting stage was reduced by 33 and 7 %, respectively, if compared to the full-irrigated conditions (Figure 4).

The main effects of foliar spraying and irrigation on grain yield per unit area were significant. The seed yields of plants grown under I2, I2 and I, were 38, 26 and 6 % lower, respectively, when compared to full-irrigation. Although the foliar application of growth stimulants slightly improved the grain yield, in relation to the control, no significant difference was seen between the solutions. The I × S effects were statistically significant at the level of 1 % on the percentage of seed oil. The results of the present experiment showed that, under favorable soil moisture conditions, the sprays were able to significantly improve the biosynthesis or accumulation of oil in the seed. All plants treated with growth stimulants or nanoparticles (S<sub>1</sub>-S<sub>4</sub>) showed the highest percentage of oil (average of 41.86 %) under full-irrigation conditions. The lowest percentage of oil was recorded under rainfed

conditions without foliar spraying (31.70 %). The reason for this could be the change in the partitioning of photoassimilates towards the pathways of fatty acid and oil biosynthesis after foliar spraying (Nath et al. 2016). However, for the plants grown under rainfed conditions,  $I_3$  and  $I_4$  caused decreases of 9, 7.2 and 3.6 % in the oil percentage, respectively. The highest improvement effect of the foliar spraying treatments on oil percentage was achieved with the application of nano zinc (37.65 %) and thiamine (37.60 %) (Figure 5).

The principal component analysis, which is used to reveal the hidden relationships among the evaluated factors, indicated that the first component was able to distinguish the optimal conditions of irrigation from the conditions of severe drought stress (rainfed and interruption of irrigation during the flowering stage). The second component was able to separate the foliar treatments with better effects from others, i.e., zinc nanoparticles, thiamine and ascorbic acid. The most effective foliar treatments were recorded under full-irrigation (Figure 6).

The clustering of traits according to the behavioral similarity against the evaluated treatments (irrigation and foliar spraying) indicated that traits such as chlorophyll, relative leaf water content,

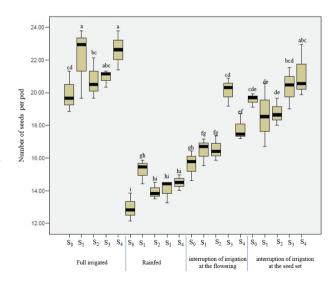


Figure 4. Effect of irrigation regimes and drought stress and spraying of growth stimulants on the number of seeds per pod in rapeseed grown in the semi-arid region of Qazvin (Iran). S<sub>0</sub>: foliar spray with distilled water; S<sub>1</sub>: foliar spray with thiamin (250 ppm); S<sub>2</sub>: foliar spray with ascorbic acid (1 mM); S<sub>3</sub>: foliar spray with silicon dioxide nanoparticles (100 ppm); S<sub>4</sub>: foliar spray with zinc oxide nanoparticles (500 ppm).

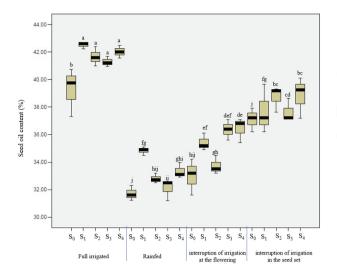


Figure 5. Mean comparison of the seed oil percentage under irrigation regimes and interruptions at different reproductive stages, as well as spraying with growth stimulants, on rapeseed in the semi-arid region of Qazvin (Iran). S<sub>0</sub>: foliar spray with distilled water; S<sub>1</sub>: foliar spray with thiamin (250 ppm); S<sub>2</sub>: foliar spray with ascorbic acid (1 mM); S<sub>3</sub>: foliar spray with silicon dioxide nanoparticles (100 ppm); S<sub>4</sub>: foliar spray with zinc oxide nanoparticles (500 ppm).

number of seeds per pod, oil percentage and 1,000-seed weight were placed in the cluster 1. The highest amount of the mentioned traits was obtained by using zinc nanoparticles under full-irrigation. In the second cluster were located traits such as days to maturity, plant height, seed yield, aboveground biomass and number of pods per plant, showing their best performance with a foliar spray of ascorbic acid under full-irrigation. The canopy width and number of branches per plant were classified in the third cluster, and the effect of thiamine on these traits was more evident than other growth stimulants (Figure 7).

The meteorological data of the studied area (temperature and rainfall) confirmed that the air temperature increased abruptly approximately at the beginning of the rapeseed reproductive growth, and the rainfall also showed a significant decrease. However, a detailed survey in the region has shown that the potential evaporation and transpiration from the grass surface is about 1,250 mm, and the transpiration in winter crops under standard conditions is more than 500 mm during the growing season (Ebrahimipak et al. 2018). These results indicate that the water shortage and the occurrence

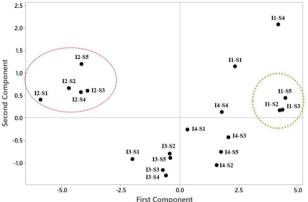


Figure 6. Principal component analysis (PCA) showing a plot of the first two PCs for all combined treatments (irrigation regimes and foliar spray treatment) on rapesced grown in the Qazvin region (Iran). I<sub>1</sub>: full-irrigation; I<sub>2</sub>: rainfed condition; I<sub>3</sub>: interruption of irrigation at the flowering stage; I<sub>4</sub>: interruption of irrigation at the seed setting stage. S<sub>1</sub>: foliar spray with distilled water; S<sub>2</sub>: foliar spray with thiamin (250 ppm); S<sub>3</sub>: foliar spray with ascorbic acid (1 mM); S<sub>4</sub>: foliar spray with silicon dioxide nanoparticles (100 ppm); S<sub>5</sub>: foliar spray with zinc oxide nanoparticles (500 ppm).

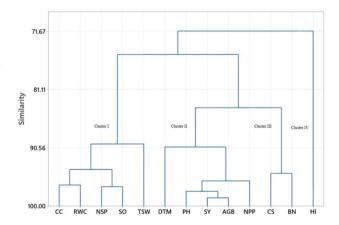


Figure 7. Cluster of the rapeseed morphophysiological traits according to the behavioral similarity against irrigation and spraying treatments with growth stimulants. DTM: days to maturity; CC: chlorophyll content; RWC: leaf relative water content; NPP: number of pods per plant; NSP: number of seeds per pod; SO: seed oil percentage; PH: plant height; AGB: aboveground biomass; CS: canopy spread; BN: number of branches; TSW: 1,000-seed weight; SY: seed yield; HI: harvest index.

of dry spells at the end of the development period in the region are inevitable.

Climatic conditions in the region during the investigated season were such that the source-sink

relationships were strongly affected. For rapeseed, the seed yield is constructed from current photosynthesis (recently acquired photoassimilates at post-anthesis growth stages), as well as photoassimilates that were produced earlier during the vegetative phase and stored in vegetative organs such as stems (Wang et al. 2023). The lowest yield was obtained under rainfed conditions. The total rainfall during the growing season was 250 mm, and, despite one irrigation during the seed sowing, the soil moisture for the standard evapotranspiration was not provided. It seems that the unequal distribution of rainfall, predominant rainfall during the winter when it coincides with the inactive growth of plants, insufficient storage of moisture in the soil, low soil water holding capacity, unsuitable soil physical conditions and high potential for evapotranspiration are the main reasons for the significant decrease of the seed yield under rainfed conditions.

Considering the source-sink relationships, it seems that rainfed conditions and irrigation interruption at the flowering stage not only decreased the current photosynthesis during the reproductive stage and the formation of yield components, but also the photoassimilates stored in the vegetative organs and remobilization process were not adequate to prevent the seed yield decline. Rapeseed under drought stress conditions is faced with serious limitations in source-sink relationships, and it may be source-limited or source-and-sink co-limited (Zhang & Flottmann 2018). The study showed that canopy width was one of the most important traits in rapeseed, which showed a high correlation with seed yield and oil percentage. This component was strongly influenced by irrigation treatments and to some extent by foliar spraying treatments. The lowest canopy width was recorded under rainfed conditions. It may show that the soil water deficit has greatly reduced the size of the source and the amount of produced photoassimilates. The most obvious effects of interruption of irrigation on this component were observed at the flowering stage. Although it was thought that the canopy width had been completed during the flowering stage, the irrigation termination at this stage greatly reduced this component. In rapeseed, the flowering stage usually coincides with the largest leaf area and photosynthetic organs (Aboodeh et al. 2024). Our results showed that stopping irrigation during the flowering stage caused a sharp decrease in

the chlorophyll content and accelerated the leaf senescence and maturity. Drought stress can accelerate the plant aging process by changing the ratio of phytohormones and increasing abscisic acid (Ahluwalia et al. 2021). Also, derogating the photosynthetic pigments and restricting the RubisCO enzyme cause a decrease in the current photosynthesis (Wahab et al. 2022). Although the interruption of irrigation at the seed setting stage slightly reduced the grain yield, it shows that the current photosynthesis was not enough to prevent the yield reduction by compensating for the lost photosynthesis. In other words, the limitation of the source and deficiency of photoassimilates to fill the seeds was evident, even under I<sub>4</sub>.

The oil percentage decreased under drought stress conditions, what may be attributed to the reduction in the precursors for the production of fatty acids and the inhibition of some enzymes involved in the oil biosynthesis pathway, such as acetyl-CoA carboxylase, FA synthase and glycerol-3-phosphate acyltransferase. Also, an increased breakdown of fatty acids may play a significant role in reducing the oil percentage under drought stress (Li et al. 2021). The effect of the foliar spraying treatments was not significant for improving yield, when compared to the irrigation treatments. Probably, the low effect of the foliar spraying treatments was caused by inappropriate concentrations or application at incorrect stages. The effect of applying foliar treatments is highly dependent on environmental conditions; however, some treatments were able to have positive effects. Under full-irrigation conditions, the foliar application of zinc, thiamine and ascorbic acid nanoparticles improved the measured traits to some extent.

Soils in semi-arid areas are often deficient in zinc (Younas et al. 2023). The supply of zinc nanoparticles has probably been able to provide part of the plant's need for zinc. Since zinc plays a key role in biosynthesizing enzymes of amino acids such as tryptophan, phytohormone auxin, and regulating the biosynthesis of pigments and the photosynthesis speed (Nazir et al. 2021), such improvement effects were expected with zinc foliar spraying. The thiamine and ascorbic acid probably improved the growth and performance of rapeseed by stimulating the defense systems, scavenging reactive-oxygen species and maintaining the relative leaf water content (Akram et al. 2017, Fitzpatrick & Chapman 2020). The

findings showed that the rapeseed flowering stage in the studied area is very sensitive to water shortage, due to the low rainfall and high evapotranspiration. Considering the amount of rainfall in the region and the amount of evaporation and transpiration, it seems necessary to use two supplementary irrigations during the flowering and seed setting stages under rainy conditions.

Although there is not much evidence about the detailed partitioning of photoassimilates between the source and sink organs in rapeseed (Smith et al. 2018), the water shortage stress during the vegetative growth period and flowering stage affected both the source (vegetative characteristics such as plant height, canopy width, chlorophyll content and branch number) and the sink (number of pods and number of seeds per pod). Furthermore, it has been indicated that, under drought stress conditions, fluctuations occurred in the total fat content, especially changes in the composition of fatty acids and in the metabolic pathway of erucic acid (for example, oleic, gadoleic and erucic acids) (Bouchereau et al. 1996). The obtained results also indicated a very significant effect of drought stress on seed oil content; however, the foliar application had less effect on seed percentage. Beneficial nanoparticles were able to improve the percentage of seed oil to some extent. It seems that part of this increase is due to the improvement of carbon supply for the biosynthesis of fatty acids in the seed. Winter cultivation and irrigation at the end of the growing season improve the oil percentage and quality by improving the source capacity and supply of photoassimilates (Safavi Fard et al. 2018). The obtained results showed that the effect of continuous water-deficit stress under rainfed conditions on yield components was much more evident than the vegetative growth. The flowering stages in rapeseed in the investigated areas were highly sensitive to drought stress. Therefore, it seems that the management of crop rotations, preventing the simultaneous irrigation of crops and planning for sufficient water supply at this stage are the most climate-smart agriculture practices to mitigate the effects of climate change (Janmohamadi & Sabaghnia 2023). However, this result is in accordance with a previous study which demonstrated that zinc nanoparticles improved the biochemical, nutritional and antioxidative capacity, as well as the fatty acid profile, of rapeseed (Sohail et al. 2020).

#### CONCLUSIONS

- 1. Stopping irrigation during the flowering stage significantly reduced all traits related to yield components;
- 2. Drought stress at the end of the reproduction and seed formation stage, when compared to drought stress at the flowering stage, caused a relatively smaller decrease in crop yield;
- 3. Considering the cost of very scarce water resources in the studied area and crop yield loss, the interruption of irrigation during the seed setting stage of winter rapeseed can be considered a climate-smart agriculture practice;
- 4. Foliar spraying, especially with zinc nanoparticles and drought tolerance improvers, can reduce the effects of water shortage stress to some extent in drought-prone areas. However, the foliar application of zinc nanoparticles, ascorbic acid and thiamine under full-irrigation conditions could improve vegetative growth and yield components.

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