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# Effect of Zeolite and Zinc on the Biochemical Characteristics of Canola upon Drought Stress

(Kesan Zeolit dan Zink ke atas Ciri Biokimia Canola Akibat Tekanan Kemarau)

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ABSTRACT

*The effects of zeolite and zinc foliar applications on the biochemical characteristics of canola cultivars under different moisture regimes were investigated in a study conducted during the 2010 and 2011 growing seasons. The study was completed using a factorial split-plot experiment based on randomized complete block design (RCBD) with three replications at the Seed and Plant Improvement Institute (SPII), Karaj, Iran. The treatments were: irrigation (I): complete ($I_1$) and restricted ($I_2$); zeolite (Z): 0 ($Z_1$) and 15 ton ha$^{-1}$ ($Z_2$) and Zn: 0, 0.1 and 0.2% concentrations of zinc sulfate ($Zn_1$, $Zn_2$, and $Zn_3$) at the pod formation stage. These treatments were applied during the pod formation stage to the Licord, RGS003 and Opera cultivars. This study showed that although applying Z and Zn had positive effects on the quality of canola, the highest performance and the best results were obtained using a combination of Z and Zn. The combined application of Z and Zn decreased the proline and carbohydrate contents to 44.35 and 34.42%, respectively. Therefore, with the low cost of natural Z and moderate Zn intake, these treatments can be used to enhance the performance of canola, especially in regions frequently subjected to water stress.*

*Keywords: Carbohydrate; drought; proline; zeolite; zinc*

ABSTRAK

*Kesan penggunaan zeolit dan zink berdaun pada ciri biokimia kultivar canola di bawah rejim kelembapan berbeza dikaji sepanjang musim penanaman 2010 dan 2011. Kajian ini menggunakan satu uji kaji faktorial plot-terbahagi berdasarkan reka bentuk keratan blok lengkap (RCBD) dengan tiga ulangan di Institut Penambahbaikan Benih dan Tumbuhan (SPII) di Karaj, Iran. Jenis rawatan adalah: pengairan (I): lengkap ($Z_1$) dan terhad ($Z_2$); zeolite (Z): 0 ($Z_1$) dan 15 tan ha$^{-1}$ ($Z_2$) dan Zn: kepekatan 0, 0.1 dan 0.2% zink sulfat ($Zn_1$, $Zn_2$, dan $Zn_3$) di peringkat pembentukan pod. Rawatan ini telah digunakan semasa peringkat pembentukan pod kultivar untuk Licord, RGS003 dan Opera. Kajian ini menunjukkan bahawa walaupun penggunaan Z dan Zn mempunyai kesan positif terhadap kualiti canola, prestasi tertinggi dan keputusan terbaik diperoleh menggunakan kombinasi Z dan Zn. Gabungan Z dan Zn menurunkan kandungan prolin dan karbohidrat masing-masing kepada 44.35 dan 34.42%. Oleh itu, dengan kos rendah Z semula jadi dan pengambilan sederhana Zn, rawatan ini boleh digunakan untuk meningkatkan prestasi canola, terutamanya di kawasan-kawasan yang kerap tertakluk kepada tekanan air.*

*Kata kunci: Karbohidrat; kemarau; prolin; zeolit; zink*

## INTRODUCTION

Drought is the most important environmental stress affecting agriculture worldwide (Yang et al. 2010). In recent years, many studies on the effects of complementary irrigation on crop yield and water use efficiency (WUE) have shown that adequate complementary irrigation can increase crop yield by improving soil moisture conditions and WUE significantly (Deng et al. 2002). According to Lessani and Mojtahedi (2006), the ability of plant cells to survive intensive water loss without experiencing nocuous damage is a core aspect of drought tolerance.

Canola (*Brassica napus* L.) is one of the most important oil crops severely affected by drought. Under late-season drought stress, canola oil yield is decreased during the flowering and seed-filling stages. Studies have shown that seed filling, pollination and flowering are stages sensitive to drought stress in many plants (Thomas et al. 2004). Masoud Sinaki et al. (2007) found that the maximum yield reduction of canola was obtained when water stress occurred at the pod developmental stage.

Anjum et al. (2013) reported that the chlorophyll (Chl) content of soybean plants was reduced under drought stress conditions. Rahman et al. (2004) stated that Chl pigments in corn were destroyed more quickly with increasing intensity of drought stress. The available water in the soil is an important factor that increases crop yields (Ghooshchi et al. 2008). One of the ways to increase the available water in the soil is by applying superabsorbent polymers that supply water to crop roots (Pawlowski et al. 2009). Under normal irrigation and water stress conditions, the desired plant yield in terms of better growth and enlargements can be achieved via the use of superabsorbent polymers that often increase the capacity for water storage in soil (Akhter et al. 2004; El-Hady & Wanas 2006; Sarvas et al. 2007). Reduction in waste water and nutrition materials in soil (Adams & Lockaby 1987), as well as in soil surface water evaporation (Akhter et al. 2004; Sarvas et al. 2007; Sivapalan 2001) and increased soil aeration (Orzeszyna et al. 2006) are among the significant contributions of such absorbents towards the generation of the desired plant yield. These materials can be used to decrease the irrigation frequency by increasing the gaps between irrigations, thereby saving on water cost and energy (Sivapalan 2001). The application of natural zeolite (Z) in agricultural production and environmental protection as a superabsorbent polymer has increased because of its high absorption capacity and cation exchanging capacity (CEC) (Shirani Rad 2011).

The application of Z into the soil increases the water accumulation capacity. Z acts as a chemical sieve that allows some ions to pass through while blocking others (Ok et al. 2003). The high CEC of Z reduces the leaching of nutrients, particularly nitrates and plays a significant role in agriculture (Zahedi et al. 2009). The selective uptake and regulated diffusion of nutrients by Z can help plants overcome deficiencies (Masoud Sinaki et al. 2007). The unique characteristics of Z, including high CEC, selective uptake, inexpensiveness, supply abundance and structural stability makes it an excellent material for soil reinforcement to enhance the drought stress tolerance of soil and optimize fertilizer use (Ok et al. 2003).

Eskandari Zanjani et al. (2012) results indicated that drought stress caused significant physiological and biochemical changes in medicinal pumpkin. Z application under drought stress conditions increased total Chl. However, proline accumulation was reduced in the presence of Z under drought stress conditions compared with the control treatment.

Water deficiency affects the efficiency of fertilizers. Zinc sulfate has an important role in the plant system to decrease water stress, such that any secondary factor leading to the inaccessibility of this element for the plant, affects the yield and concentration of this element in various tissues (Khurana & Chatterjee 2001). Ghasemian et al. (2010) showed that Zn application yielded maximum number of seeds per plant in soybean. Zn foliar application increases tryptophan amino acid and indol acetic acid hormone (IAA), which are the two main factors of leaf area expansion in *Phaseolus vulgaris* (Seifi Nadergholi et al. 2011). Therefore, under water deficiency conditions, the use of fertilizers should be balanced and the consumption of fertilizers such as zinc sulfate has to be considered. The present study was conducted to assess the effects of Z and Zn applications on the biochemical characteristics of canola cultivars under different moisture regimes.

## Materials and Methods

The effects of natural Z and Zn on the physiological characteristics of canola varieties under different moisture regimes was investigated during the 2010 and 2011 growing seasons. The average annual rainfall (based on 30 years of rainfall records) was 244 mm (Figure 1), which occurs mainly during the fall and winter months (November to February). This study was conducted using a randomized complete block design (RCBD) in a factorial split-plot experiment with three replications at the Seed and Plant Improvement Institute (35° 59′ N, 50° 75′ E, with an altitude of 1313 m), Karaj, Iran. The seeds were disinfected and sown in early October (2010 and 2011). Each experimental plot consisted of six cultivation lines 0.3 m apart, with a population of 100 shrubs for every square meter for a length of 5 m. The blocks were separated by 6 m distance to avoid margin effects. Every block was set at a distance of 2.4 m from the main plots. Irrigation was performed uniformly across the plots until the pod formation stage. The treatments were: irrigation (I): normal irrigation (control) ($I_1$), and restricted (interruption of irrigation at pod formation stage) ($I_2$); Z: 0 ($Z_1$), and 15 ton ha$^{-1}$ ($Z_2$) and Zn: 0, 0.1 and 0.2% concentrations of zinc sulfate ($Zn_1$, $Zn_2$ and $Zn_3$) applied on the Licord, RGS003 and Opera cultivars. Zeolite and chemical fertilizers were applied and incorporated into the soil. The foliar application of Zn as zinc sulfate was conducted during the pod formation stage in three concentrations (0, 0.1, and 0.2%) using an engine backpack sprayer. Weed control was done by hand.

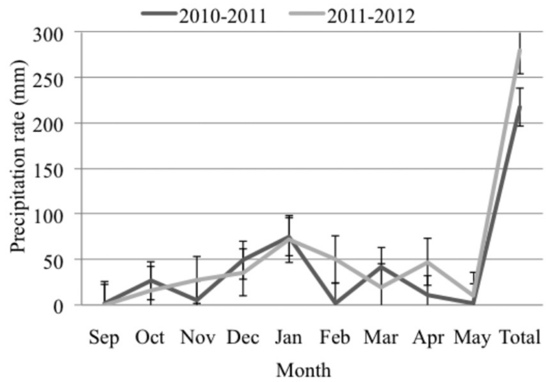



FIGURE 1. Monthly precipitation rate (mm) in 2010-2012 cropping seasons at experiment site

Mixed soil samples were collected from the depths of 0 to 30 cm and 30 to 60 cm to determine the physicochemical parameters (Table 1).

### Sampling

After drought stress treatment, samples from the middle leaves of each treatment (the third leaf from the apex of the plant) was harvested. Bulk fresh tissues were collected, washed and frozen in liquid $N_2$ and then stored at -80°C until biochemical analysis (Eskandari Zanjani et al. 2012; Tohidi Moghadam et al. 2012).

TABLE 1. Physicochemical properties of soil collected from site study

| Year | Depth (cm) | EC (ds.m-1) | pH | Organic carbon (%) | Saturated percentage (%) | N (%) | P (ppm) | K (ppm) | Fe (ppm) | Cu (ppm) | Zn (ppm) | T.N.V (%) | Texture |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2010 | 0-30 | 1.32 | 7.8 | 0.49 | 30.63 | 0.05 | 4.7 | 174 | 5.5 | 7.3 | 0.84 | 8.55 | Clay |
| | 30-60 | 1.73 | 7.7 | 0.36 | 30.85 | 0.04 | 2 | 133 | 2.2 | 3.9 | 0.51 | 10.49 | loam |
| 2011 | 0-30 | 1.41 | 7.8 | 0.51 | 35.96 | 0.08 | 3.1 | 208 | 5.2 | 7.1 | 0.89 | 9.86 | Clay |
| | 30-60 | 1.42 | 7.9 | 0.39 | 37.09 | 0.06 | 2 | 148 | 2.1 | 3.6 | 0.47 | 10.29 | loam |

### CHLOROPHYLL CONTENT ASSAY

Leaf samples (1 g) were extracted in 10 mL 100% acetone using a mortar and pestle. The homogenate was filtered and centrifuged at 2000 rpm for 2 min. A 1 mL aliquot of the supernatant was pipetted and mixed with 9 mL of 80% acetone. The absorbance of the diluted samples was obtained using a spectrophotometer at 663 and 646 nm. Chl *a*, Chl *b* and total Chl were calculated according to the following equations (Ianculov et al. 2005):

$$\text{Chl } a = 12.21 \cdot A_{663} - 2.81 \cdot A_{646}.$$

$$\text{Chl } b = 20.13 \cdot A_{646} - 5.03 \cdot A_{663}.$$

$$\text{Chl}_{total} = 7.32 \cdot A_{646} + 7.18 \cdot A_{663}.$$

where, Chl *a* is the chlorophyll *a*, in mg $L^{-1}$ , Chl *b* is the chlorophyll *b*, in mg $L^{-1}$ , $\text{Chl}_{total}$ is the total chlorophyll content, in mg $L^{-1}$, $A_{663}$ is the sample absorbance at 663 nm and $A_{646}$ is the sample absorbance at 646 nm.

### PROLINE ASSAY

The proline content of leaves was determined using a modified version of the method described by Bates et al. (1973). Leaf samples (0.5 g) were homogenized in a mortar and pestle with 10 mL of 3% sulfosalicylic acid and centrifuged at 3000 rpm for 15 min. A 1 mL aliquot of the supernatant was added into a test tube, into which, 1 mL glacial acetic acid and 1 mL acid ninhydrin solution were also added. The test tube was incubated in a water bath for 1 h at 100°C and allowed to cool to room temperature. Toluene (4 mL) was added to the tube and then mixed in a vortex mixer for 20 s. The test tube was allowed to stand undisturbed for at least 10 min to allow separation of the toluene and aqueous phases. The toluene phase was carefully pipetted into a glass test tube and its absorbance was measured with a spectrophotometer at 520 nm. The proline content was calculated based on a standard curve.

### TOTAL SOLUBLE CARBOHYDRATE ASSAY

Soluble carbohydrate was estimated according to the method proposed by Dubois et al. (1956). Leaf samples were homogenized in a mortar and pestle with 3 mL distilled water and the homogenate was filtered using a filter paper. Phenol (0.5 mL, 5%) and 2.5 mL of 98% sulfuric acid were added to the homogenate. After the reaction of the materials, the test tubes were allowed to cool to room temperature. The carbohydrate content was determined from the absorbance measured at 483 nm and calculated based on a standard curve.

## RESULTS AND DISCUSSION

The results of ANOVA indicated that the simple effects of the treatments (I, Zn, and Z) were statistically significant for all assessed traits at $p<0.01$ except for Zn, which did not significantly affect Chl *a*, Chl *b* and total Chl (Table 2). The biochemical characteristics of the canola cultivars responded to changes in irrigation regimes, as well as to various Z and Zn treatments in different ways (Table 3).

Restricted irrigation enhanced the proline and carbohydrate contents to 99.4 and 57.09%, respectively and decreased the Chl content to 59.84% (Table 3). Manivannan et al. (2007) reported that drought stress significantly decreased the Chl *a*, *b* and total Chl contents in sunflower. The Chl content has a close negative correlation with water stress; thus, Chl measurements can be a useful index in determining the stress intensity (Shen et al. 2008). Enhanced proline accumulation during stress indicates that proline may play a crucial role as an osmoregulatory solute in plants (Eskandari Zanjani et al. 2012). Tohidi Moghadam et al. (2012) illustrated the significant effects of water stress on the soluble carbohydrates in canola leaves.

The application of Z increased the Chl content to 22.6% but decreased the proline and carbohydrate contents to 31.49 and 28.17%, respectively, compared with the untreated groups (Table 3). Z with high CEC acts as a sink for nutrients such as ammonium, thereby improving plant growth (Polat et al. 2004). Water can penetrate easily into the Z structure. Furthermore, Z application increases the water retention capacity of the soil (Rehakova et al. 2004).

The application of Zn decreased the proline and carbohydrate contents to 6.6 and 3.52%, respectively (Table 3) and increased root growth (Khurana & Chatterjee 2001), oxine biosynthesis, Chl concentration, as well as N and phosphorous uptake (Malekoti & Tehrani 1999; Moinuddin & Imas 2008). Therefore, under water

TABLE 2. Summary of combined F significance from analysis of variance

| S.V | DF | PRO | CAR | CHLA | CHLB | CHL |
|---|---|---|---|---|---|---|
| Y | 1 | ** | ** | ns | ns | ns |
| R(Y) | 4 | ns | ns | ns | ** | * |
| I | 1 | ** | ** | ** | ** | ** |
| Zn | 2 | ** | ** | ns | ns | ns |
| Z | 1 | ** | ** | ** | * | ** |
| I*Zn | 2 | ns | ns | ns | ns | ns |
| I*Z | 1 | ** | ** | ns | ** | ** |
| I*Y | 1 | ns | * | ns | ns | ns |
| Zn*Y | 2 | ns | ns | ns | ns | ns |
| Z*Y | 1 | ns | ns | ns | ns | ns |
| Zn*Z | 2 | ** | * | ns | ns | ns |
| I*Zn*Z | 2 | ns | ns | ns | ns | ns |
| I*Zn*Y | 2 | ns | ns | ns | ns | ns |
| I*Z*Y | 1 | ns | ns | ns | ns | ns |
| Zn*Z*Y | 2 | ns | ns | ns | ns | ns |
| I*Zn*Z*Y | 2 | ns | ns | ns | ns | ns |
| C | 2 | ns | ns | ns | ns | ns |
| R Squared | | 0.991 | 0.985 | 0.903 | 0.562 | 0.850 |

*Y*: year, *I*: Irrigation, *Z*: Zeolite, *Zn*: Zinc, *C*: Cultivar, *R*: Replication
*PRO*: Proline, *CAR*: Carbohydrate, *CHLA*: Chlorophyll a, *CHLB*: Chlorophyll b, *CHL*: Chlorophyll
ns: Non-significant , * and **: Significant at 5% and 1% probability levels, respectively

TABLE 3. Mean comparisons of I, Z and Zn on biochemical characteristics

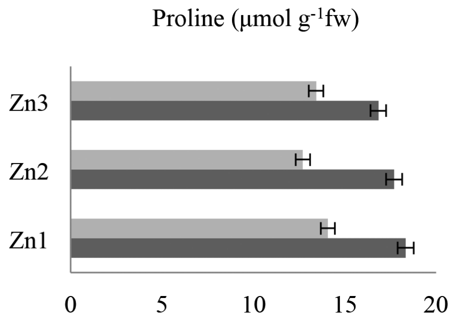
| Treatments | Mean | | | | |
|---|---|---|---|---|---|
| | PRO ($\mu$mol $g^{-1}$fw) | CAR (mg $g^{-1}$fw) | CHLA (mg $g^{-1}$fw) | CHLB (mg $g^{-1}$fw) | CHL (mg $g^{-1}$fw) |
| Irrigation | | | | | |
| $I_1$ | 10.6125b | 29.1844b | 1.0767a | 0.6667a | 1.7434a |
| Std. Error | 0.14898 | 0.36458 | 0.01255 | 0.03243 | 0.03285 |
| $I_2$ | 20.4207a | 45.8481a | 0.6771b | 0.4402b | 1.1173b |
| Std. Error | 0.29761 | 0.58946 | 0.01542 | 0.01542 | 0.01323 |
| Zeolite | | | | | |
| $Z_1$ | 176257a | 42.1484a | 0.7864b | 0.5067b | 1.2913b |
| Std. Error | 0.55804 | 0.92590 | 0.02105 | 0.02084 | 0.03207 |
| $Z_2$ | 13.4056b | 32.8841b | 0.9687a | 0.6003a | 1.5690a |
| Std. Error | 0.40695 | 0.72082 | 0.02295 | 0.03338 | 0.04328 |
| Zinc | | | | | |
| $Zn_1$ | 16.2092a | 38.4104a | 0.8668a | 0.5646a | 1.4314a |
| Std. Error | 0.65993 | 1.18248 | 0.02449 | 0.02894 | 0.04062 |
| $Zn_2$ | 15.2053b | 37.1032b | 0.8897a | 0.5688a | 1.4585a |
| Std. Error | 0.66205 | 1.16700 | 0.02474 | 0.02721 | 0.04084 |
| $Zn_3$ | 15.1352b | 37.0345b | 0.8892a | 0.5616a | 1.4508a |
| Std. Error | 0.61856 | 1.11448 | 0.02320 | 0.03074 | 0.04209 |

Any two means sharing a common letter do not differ significantly from each other at 5% probability
*PRO*: Proline, *CAR*: Carbohydrate, *CHLA*: Chlorophyll a, *CHLB*: Chlorophyll b, *CHL*: Chlorophyll

deficiency conditions, the use of fertilizers should be balanced and the consumption of special fertilizers such as zinc sulfate has to be especially considered.

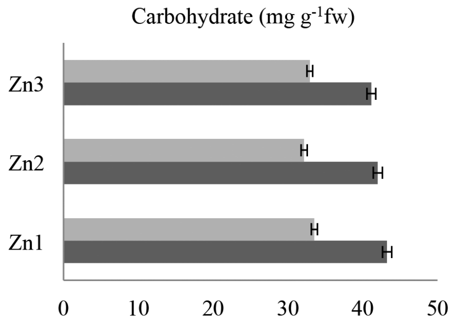
The effects of the interaction between Z and Zn had different effects on the proline ($p<0.01$) and carbohydrate ($p<0.05$) contents but had similar effects for Chl *a*, *b* and total Chl (Table 2). Studies on the effects of the interaction between Z and Zn on biochemical characteristics indicated that the lowest proline and carbohydrate accumulation rates were obtained with the application of $Z_2Zn_2$ (15 ton $ha^{-1}$ Z and 0.1% zinc sulfate) was applied. The combined application of Z and Zn decreased the proline and carbohydrate accumulation rates to 44.35 and 34.42%, respectively, compared with the untreated groups (Figures 2 and 3).

The effects of the interaction between I and Z were significant for all studied traits at $p<0.01$, except for Chl *b* (Table 2). A study on the effects of the interaction between I and Z on biochemical characteristics (Figures 4 to 6)




| | Zn1 | Zn2 | Zn3 |
|---|---|---|---|
| Z2 | 14.075 | 12.707 | 13.435 |
| Z1 | 18.343 | 17.704 | 16.836 |

FIGURE 2. Interaction effects of Z and Zn on proline content
Error bars indicate SE ($n$=3)




| | Zn1 | Zn2 | Zn3 |
|---|---|---|---|
| Z2 | 33.554 | 32.186 | 32.912 |
| Z1 | 43.266 | 42.021 | 41.158 |

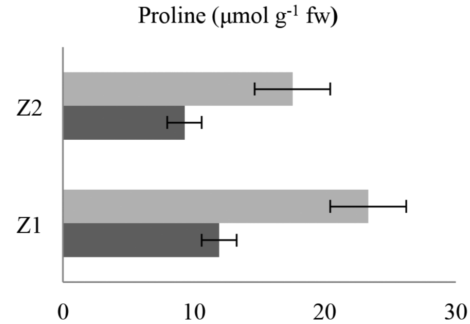
FIGURE 3. Interaction effects of Z and Zn on carbohydrate content
Error bars indicate SE ($n$=3)

indicated that the highest Chl content and lowest proline and carbohydrate accumulation rates were obtained upon the application of $I_1Z_2$ (normal irrigation and 15 ton ha$^{-1}$ Z). Shirani Rad (2012) reported that the normal irrigation and Z consumption of 15 ton ha$^{-1}$ produced the highest Chl *a*, Chl *b* and total Chl contents. The accumulation of osmolyte compounds such as sugars and amino acids, particularly proline, in the cells as a result of water stress is often associated with a possible mechanism for tolerating the harmful effects of water shortage (Pirzad et al. 2011).

No statistically significant difference was observed among the cultivar types (Table 3). Similar results were found when two canola cultivars compared in terms of proline and Chl content (Tohidi Moghadam et al. 2012). Bannayan et al. (2008) reported that the effects of water stress on growth and yield depend on plant genotype.

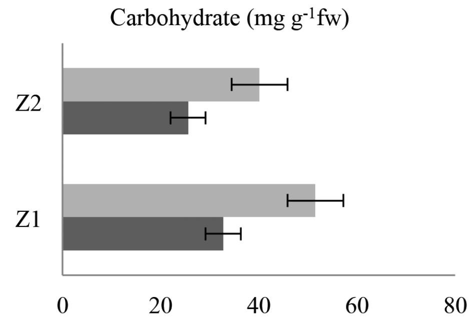
## CONCLUSION

The study showed that the biochemical characteristics of canola cultivars responded to changes in irrigation regimes, as well as to various Z and Zn treatments in different ways. The highest Chl content and the lowest proline and carbohydrate content were obtained with the application of $I_1Z_2$ (normal irrigation and 15 ton ha$^{-1}$



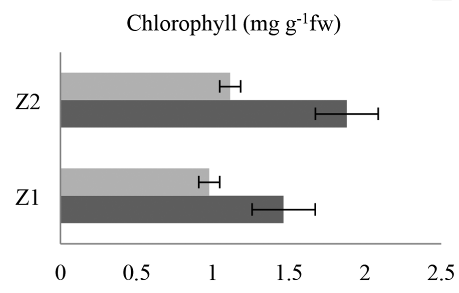

| | Z1 | Z2 |
|---|---|---|
| I2 | 23.317 | 17.525 |
| I1 | 11.938 | 9.287 |

FIGURE 4. Interaction effects of I and Z on proline content
Error bars indicate SE ($n$=3)




| | Z1 | Z2 |
|---|---|---|
| I2 | 51.538 | 40.158 |
| I1 | 32.759 | 25.61 |

FIGURE 5. Interaction effects of I and Z on carbohydrate content
Error bars indicate SE ($n$=3)




| | Z1 | Z2 |
|---|---|---|
| I2 | 0.976 | 1.115 |
| I1 | 1.465 | 1.88 |

FIGURE 6. Interaction effects of I and Z on chlorophyll content
Error bars indicate SE ($n$=3)

Z) and $Z_2Zn_2$ (15 ton ha$^{-1}$ Z and 0.1% zinc sulfate) respectively. This study showed that the application of Z and Zn had significant positive effects on the biochemical characteristics of canola. However, the highest performance and the best results were obtained with the application of a combination of Z and Zn. Therefore, with the low cost of natural Z and moderate Zn intake, these treatments can be used to enhance the performance of canola, especially in regions frequently subjected to water stress.

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