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Effect of Animal Manure and Trace Element on The Growth of *Camelina sativa* under Drought Stress in the West of Iran

Mohsen Janmohammadi[1]*, Hasan Kouchakkhani[1], Asghar Ebadi-Segherloo[2] and Naser Sabaghnia[1]

[1]*Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, Maragheh, P.O. Box 55181-83111, Iran*

[2]*Moghan College of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, 1136756199, Iran*

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Abstract: *Camelina sativa* is a relatively new oilseed crop adapted to semi-arid conditions. The present experiment was conducted to evaluate the effects of different levels of animal manure application (0, 10 and 20 t ha^{-1}, abbreviated as AM_0, AM_{10} and AM_{20}), as well as micronutrient applications (control, Fe, and Zn @ 7 kg ha^{-1}), on the growth and yield characteristics of camelina in the west of Iran. Plants were irrigated under deficit irrigation conditions (FC 60%) from the planting stage to the beginning of reproductive growth, and with full supplementary irrigation during the reproductive stage. The highest main stem height was achieved with AM_{20} + Zn and AM_{20} + Fe. This trend was also observed for lateral canopy growth, and the application of iron with high levels of manure increased this component by 32% compared to the control. The application of AM_{10} and AM_{20} increased the chlorophyll content of leaves by 5% and 20% compared to the control. The highest silique number was obtained under AM_{20} + Fe conditions, while the application of zinc under AM_0 conditions resulted in the highest number of seeds in silique. The heaviest seeds were obtained with the application of AM_{10} + Zn or Fe. The highest seed yield was obtained with the application of AM_{20} + Zn (1361 kg ha^{-1}) and AM_{20} + Fe (1344 kg ha^{-1}), which showed an increase of 18% compared to the control. The results showed that there are synergistic effects between the application of manure and micronutrients. To improve the effectiveness of Fe and Zn fertilizers, the use of high levels of manure is recommended. The application of manure can improve the performance of camelina in semi-arid regions by improving the physical and chemical conditions of the soil and improving the water retention capacity of the soil.

Key words: Animal manure, *Camelina sativa*, trace elements.

Camelina sativa is a relatively new oilseed crop belonging to the Brassicaceae family (Alberghini *et al*., 2022). Due to its adaptability to hot, dry, and low-input conditions, it presents a promising option for oil and biodiesel production in semi-arid regions (Neupane *et al*., 2022). Typically, it is sown before the

***Correspondence**
Mohsen Janmohammadi
mjanmohammadi@maragheh.ac.ir

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onset of winter, around October or November, allowing the plant to establish roots before colder temperatures and to utilize winter or early spring moisture. Flowering generally begins approximately 60-80 days after sowing, with maturation and seed harvest occurring in late spring to early summer (May to June). The reproductive stages of most crops, including *Camelina sativa*, are particularly vulnerable to water stress (Wei *et al*., 2023). In semi-arid Iran, the majority of annual precipitation occurs between October and February, often leading to water shortages during flowering and seed maturation, which can reduce yields (Razmavaran *et al*., 2024; Kheiri *et al*., 2017). Climate change is further altering these rainfall patterns, exacerbating water scarcity during critical growth stages (Janmohammadi and Sabaghnia, 2023). To mitigate these challenges, implementing efficient irrigation scheduling and reducing water use during vegetative stages can help conserve water resources for the sensitive reproductive phases (Wabela *et al*., 2022). Research indicates that limited irrigation—providing up to 75% of crop evapotranspiration—can enhance water use efficiency in *Camelina sativa*. Moreover, mild water deficit stress during growth can activate tolerance mechanisms, improving the plant's resilience to hot and dry conditions at the end of the growing period (Neupane *et al*., 2020).

Soils in the semi-arid regions of Iran are characterized by limited vegetation cover (Eskandari Dameneh *et al*., 2021), primarily attributable to the low and highly heterogeneous distribution of precipitation. These soils are typically shallow, with low organic matter content and elevated pH levels, which adversely affect soil chemical properties. Under such conditions, cation exchange capacity (CEC) is substantially reduced, leading to decreased availability of essential nutrients for plant uptake (Zádorová *et al*., 2025). The paucity of soil organic matter further compromises soil physical properties leading to a marked decrease in water permeability and retention capacity. This deterioration promotes increased surface runoff during precipitation events, resulting in significant losses of water that bypass plant roots, thereby reducing effective soil water availability (Védère *et al*., 2022). Although *Camelina sativa* demonstrates inherent resilience under low-input agronomic conditions, improvement of soil physical and chemical properties through targeted soil management practices can substantially augment crop performance. Improvements such as organic matter augmentation and soil structure amelioration can enhance nutrient bioavailability, increase water retention, and mitigate runoff, thereby facilitating improved growth, higher yield components, and enhanced water use efficiency in semi-arid environments.

Although most farmers focus on supplying the essential NPK elements, plants require some other elements in very small amounts, and if they are not supplied, yield will be significantly reduced (Ahmed *et al*., 2024). For this reason, the supply of micronutrients in semi-arid regions should be seriously considered. Iron (Fe) is of great importance due to its role in redox activity. It plays a role in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis (ethylene, gibberellin, jasmonic acid), production and scavenging of reactive oxygen species, and defense processes (Bhat *et al*., 2024). Zinc (Zn) acts as a cofactor in many key enzymes. These enzymes are often involved in some biochemical pathways such as carbohydrate metabolism, photosynthesis, sugar-to-starch conversion, protein metabolism, auxin metabolism, pollen seed formation, biological membrane integrity, and resistance to some pathogens (Castillo-González *et al*., 2018). However, there is still little information available on the use of iron and zinc in different conditions of organic soil amendments under water deficit conditions. The present experiment was designed to study the effect of iron and zinc application on seedling emergence, plant establishment, growth, and yield components of *Camelina sativa* with different amounts of animal manure under low irrigation conditions in a region in western Iran.

Materials and Methods

The experiment was conducted on agricultural fields in Kal-Kabud village, Damaq County, Hamadan Province (35°39′ N, 49°03′6″ E, elevation 1810 m) during the 2023 - 2024 cropping season. According to the Köppen climate classification, the region has a semi-arid-temperate climate with hot summers and cold winters. The average annual temperature was 15°C, with mean maximum and minimum temperatures of 21.3°C and 5.59°C, respectively.

Table 1. Physical and chemical properties of soil from the Kal-Kaboud area collected randomly from a depth of 0-30 cm

Soil texture	N (%)	K (mg kg^{-1})	P (mg kg^{-1})	Organic carbon (%)	pH	EC (dS m^{-1})	$CaCO_3$ (%)	CEC (Cmolc kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})
Loamy clay	0.12	287	14	0.49	7.54	1.38	14	12.2	0.43	0.79

Table 2. Chemical properties and available nutrients in manure collected from a calf-rearing farm

Soil texture	N (%)	K (%)	P (%)	Ca (%)	Mg (%)	S (%)	pH	EC (dS m^{-1})	Dry Matter (%)	Organic carbon (%)	Zn (mg kg^{-1})	Fe (mg kg^{-1})
Loamy clay	2.11	1.89	0.75	1.52	0.49	0.53	7.34	14.62	25.4	63.6	42.58	50.31

Annual precipitation averaged 311 mm. The soil was classified as loamy clay, and the site was fallow in the previous year. Physical and chemical properties are summarized in Table 1.

Field trial execution: The experiment was conducted in a split plot in a randomized complete block design with three replications. The experimental treatments included three levels of animal manure application including 0, 10 and 20 t ha^{-1} (indicated as abbreviations AM_0, AM_{10} and AM_{20}) were assigned to the main plots. The chemical properties and available nutrients in manure are shown in Table 2. The initial soil tillage was carried out in October 2023, and the secondary tillage, the preparation of experimental plots, and the use of animal manure were done in February 2024. The subplots included the application of micronutrients (control or no application, iron and zinc). Each experimental block consisted of 9 plots and each experimental plot was 4 × 4 m in size. The plants were planted in rows with 50 cm spacing and 4 cm inter-plant spacing. The intermediate Soheil variety was used for planting. Seeds with a purity of 99% were obtained from Pakan Seed Institute, Isfahan, Iran. The seeds were soaked in water for 24 hours before sowing to facilitate germination and then sown in rows at a depth of 3 cm on March 18. Trace element fertilizers (TE) were applied as EDTA-chelated Zn and Fe at the recommended rate of 7 kg ha^{-1} during planting as band applications and 2 cm below the position of the camelina seed.

Irrigation: From the planting stage to the beginning of the reproductive stage, moisture was provided to the entire farm through deficit irrigation. Irrigation was done through polyethylene pipes equipped with a volume meter. Time-domain reflectometer (TDR) was used to measure soil moisture during different development periods. The soil moisture content at the field capacity point was 30.7% v/v and at the permanent wilting point was 14.5% v/v. Irrigation was repeated when soil reached 55% of available soil water under full irrigation and 45% under deficit irrigation conditions. The amount of water consumption was calculated according to Hasanuzzaman *et al.*, (2016) formula: $R = (FC_{60} \times SWC) \times BD \times RD$. Where R is the water requirement (mm), FC_{60}: is the 60% field capacity, SWC: is soil water content before irrigation (through gravimetric method), BD: is soil bulk density gcm^{-3}, RD: is the rooting depth (60 cm). From the beginning of flowering, pod growth and seed filling, the field was fully irrigated (100% FC). The total deficit irrigation during the vegetative period was 245 mm and the total full irrigation during the reproductive stage was 320 mm.

Evaluation of agronomic traits: At the early stage of flowering (BBCH= 60; First flowers open), the chlorophyll of the upper and developed leaves of Camelina was measured using a manual chlorophyll meter (SPAD 502, Konica Minolta, USA). In the physiological maturity stage of Camelina (BBCH= 89; when nearly all siliquae were ripe the crop was ready to be harvested) using arbitrarily 1-m^2 quadrate from each experimental unit, main stem height was measured. The lateral growth of the canopy was calculated by measuring the diameter of the canopy from left to right. To evaluate morphological characteristics, measurements were taken from 10 plants per plot. After harvesting Camelina plants and drying them in the oven, seed yield components such as the number of total siliquae per plant, number of seeds per siliquae, weight of 1000 seeds, yield per unit area, biomass, and harvest index (ratio of seed yield to biological yield) were calculated. A Soxhlet apparatus was used to calculate the oil percentage in the seeds of the harvested plants. For this purpose, ten grams of Camelina seeds were ground and then mixed with 600 mL of hexane. After 6 hours of extraction, the solvent was

Table 3. Variance analysis and mean comparison effects of animal manure and trace elements on growth and performance characteristics of camelina in the semi-arid region of Damaq in western Iran

Irrigation (I)	Days to emergence	Plant height (cm)	Chlorophyll content in upper leaves (SPAD unit)	Thousand-seed weight (g)	Number of seeds per silique	Above ground biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Harvest index (%).
Animal manure								
AM_0	8.86a	42.11c	35.56c	0.87b	14.41a	5002.27c	1205.62c	24.10a
AM_{10}	6.62b	44.10b	37.14b	0.92a	13.76ab	5391.29b	1264.10b	23.46b
AM_{20}	4.82c	49.44a	42.53a	0.88b	13.29b	6019.39a	1336.02a	22.19c
Trace element								
Control (TE)	7.26a	43.00c	37.72b	0.87b	13.67a	5277.32b	1240.05b	23.55a
Fe	7.01a	45.38b	39.23a	0.90a	14.04a	5554.47a	1276.15a	23.04b
Zn	6.07b	47.26a	38.72b	0.90a	13.75a	5581.36a	1289.63a	23.16b
				Significance level				
AM	**	**	**	**	ns	**	**	**
TE	*	**	*	**	ns	**	**	*
AM× TE	ns	ns	ns	**	ns	ns	*	*

AM_0, AM_{10}, and AM_{20}: application of 0, 10, and 20 t ha^{-1} animal manure, control for trace element: no use of trace element fertilizer, Zn, and -Fe: the soil application of EDTA-chelated zinc or iron fertilizers during seed planting. Ns: statistically non-significant, * and ** represent significance level at 5 and 1% respectively.

separated from the oil by rotary evaporation and stored at refrigerator temperature until chemical analysis. The oil yield was obtained by multiplying the oil percentage by the seed yield. ANOVA was performed through SAS software. Box plots were drawn through SPSS Statistics. Component analysis (PCA), and boxplots were executed by Minitab software.

Results and Discussion

The results of analysis of variance (ANOVA) indicated that the application of animal manure (AM) and trace elements (TE) had a significant effect on the number of days from planting to emergence (DTE). The use of AM_{10} and AM_{20} reduced this component by 25% and 45%. On the other hand, the use of zinc fertilizer also reduced DTE by 16% compared to the control (Table 3).

The main effects of AM and TE on main stem height were significant, and AM_{10} and AM_{20} applications increased this trait by 5% and 18% compared to the control. The applications of Fe and Zn also significantly increased plant height compared to the control. The evaluation of lateral canopy growth indicated that the interaction effects of AM × TE were significant at the 1% level. The application of Fe and Zn fertilizers under AM_0 conditions had no effect on canopy growth. Whereas, under AM_{10} conditions, the efficiency of iron use increased significantly. The highest lateral growth (29.4 cm) was recorded with AM_{20} + Fe (Fig. 1).

Evaluation of chlorophyll content of upper leaves in Camelina showed that AM application at 1% and TE application at 5% affected these components. AM_{10} and AM_{20} application increased chlorophyll content by 5% and 19%, respectively. On the other hand, iron and zinc application increased chlorophyll content by 4% and 2.6%, respectively (Table 3). Number of siliquae (NSP) is one of the most important components of seed yield in Camelina. This component was affected by the interaction effect of AM × TE ($p<0.01$). The application of TE under conditions of no soil amendment utilization did not have a significant beneficial effect on NSP. However, under AM_{10} conditions, zinc application increased this component by 25% and iron application increased the number of siliquae by 14%. However, under AM_{20} conditions, iron and zinc application improved NSP by 10% and 5%, respectively. In other words, the effectiveness of TE fertilizers increased with increasing soil amendment application (Fig. 2).

Seed weight evaluation showed that the AM × TE interaction was statistically significant ($p<0.01$). The highest 1000-seed weight was recorded under AM_{10} + Zn (0.93 g) and AM_{10} + Fe (0.927 g). The lightest seed was recorded

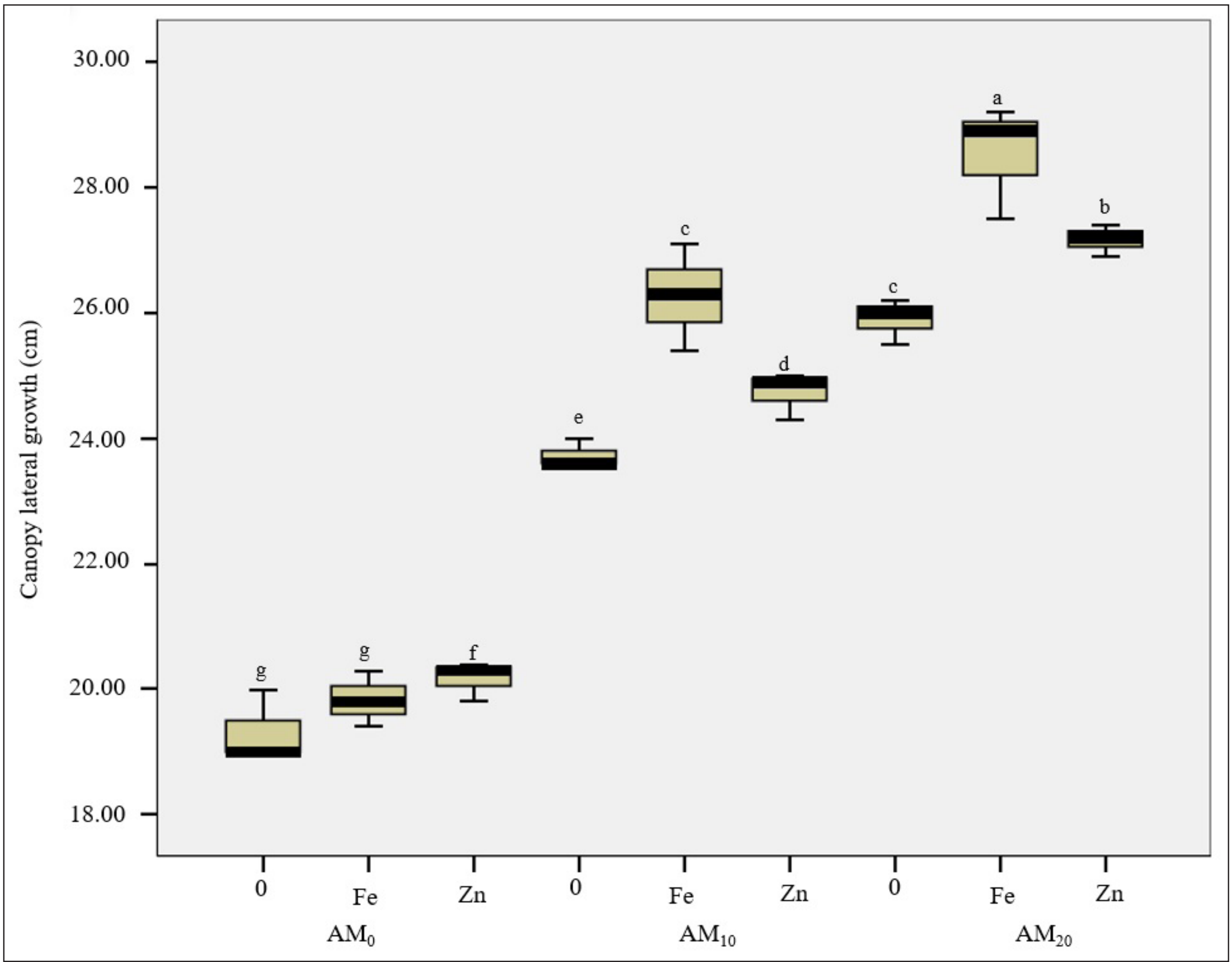

Fig. 1. The effect of animal manure (AM) and trace elements (Zn and FE) application at different levels on the lateral extent of Camelina canopy in the semi-arid region of Damaq in western Iran. Columns with different letters are statistically significant at the 5% level. AM_0, AM_{10}, and AM_{20}: application of 0, 10, and 20 t ha^{-1} animal manure, control (0) for trace element: no use of trace element fertilizer, Zn, and -Fe: the soil application of EDTA-chelated zinc or iron fertilizers during seed planting.

for plants grown under AM_{20} + C (0.85 g) and AM_0 + C (0.86 g). Among the yield components, seed number in silique was not affected by the studied treatments. ANOVA showed that AM×TE interaction was significant on seed yield (Fig. 3). The highest seed yield was recorded under the conditions of AM_{20}+Zn (1361 kg ha^{-1}) and AM_{20}+Fe (1344 kg ha^{-1}). The lowest seed yield was recorded in plants grown under the conditions without soil amendments and trace elements applications (1165 kg ha^{-1}). In this trait, it was also observed that the effectiveness of TE increases with increasing AM application.

The plant biomass study showed that the main effects of AM and TE were statistically significant at the 1% level. The application of AM_{10} and AM_{20} increased plant biomass by 8% and 20% compared to the control. On the other hand, the application of zinc and iron did not differ significantly in terms of their effect on plant biomass and increased this component by about 6% compared to the control (Table 3).

Comparisons of the means for seed yield (product of seed yield and oil percentage) indicated that plants grown under AM_{20} + Zn produced the highest oil yield (462 kg ha^{-1}). The lowest oil yield was recorded under conditions without manure and TE at about 360 kg ha^{-1}. Under AM_{10} conditions, iron application had little effect on oil yield, while with increasing use of soil amendments, the positive effect of TE also increased significantly (Fig. 4).

However, the evaluation of the harvest index showed that the AM × TE interaction was statistically significant at the 5% level. The highest harvest index was obtained in AM_0 + Fe and AM_0 + Control conditions. While the lowest

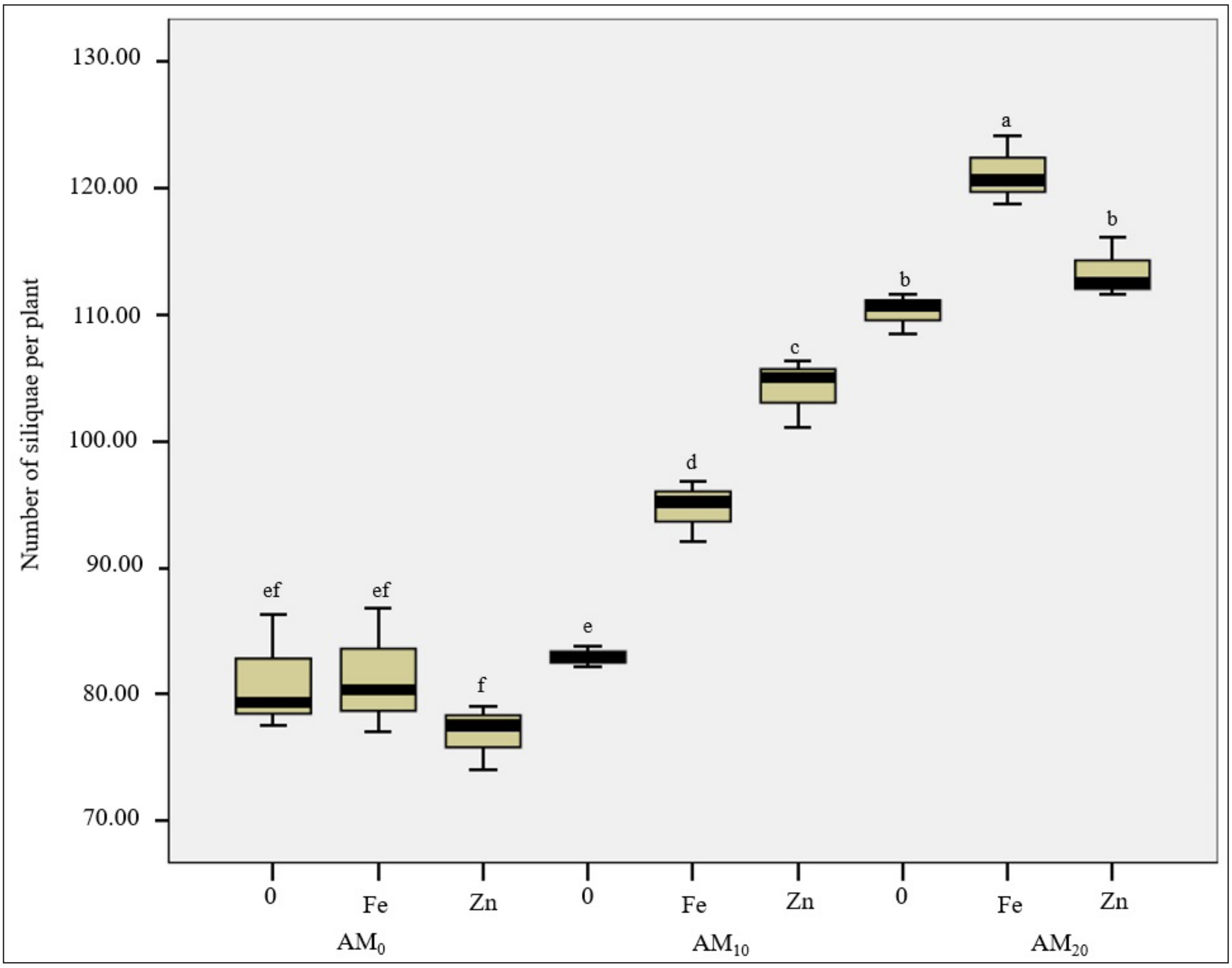

Fig. 2. The evaluation of animal manure and trace elements application on number of siliquae in camelina plants grown in semi-arid region in west of Iran. Columns with different letters are statistically significant at the 5% level. AM_0, AM_{10}, and AM_{20}: application of 0, 10, and 20 t ha^{-1} animal manure, control (0) for trace element: no use of trace element fertilizer, Zn, and -Fe: the soil application of EDTA-chelated zinc or iron fertilizers during seed planting.

harvest index was obtained in plants grown under AM_{20} + Iron and AM_{20} + Zn conditions. It seems that the use of animal manure and TE had a greater improving effect on vegetative growth. However, these studies were conducted under moisture deficiency conditions during the vegetative growth. The plot of the first and second components of the principal component analysis showed that seed yield formed a sharp angle in terms of spatial distribution with traits such as oil percentage, biomass, main stem height, and number of siliquae, which indicates a positive and significant correlation between the aforementioned traits. The greatest improving effect on yield and related traits was achieved with the application of AM_{20} + Zn and AM_{20} + Fe (Fig. 5).

The studied region had a semi-arid and temperate climate. In this region, most precipitation occurred during the cold months and early spring. Despite the use of deficit irrigation management techniques and a 40% reduction in irrigation during the vegetative growth period, only about 250 mm of water was saved compared to fully irrigated fields. However, the seed yield loss due to deficit irrigation was estimated at 21%. The aforementioned values and comparisons, as well as the high importance of water in the region, indicate the acceptability of deficit irrigation techniques and the success of water conservation. However, it seems that with some changes in irrigation programs or changes in planting date, the loss due to deficit irrigation can be reduced. The aforementioned planting was done in spring. Changing the planting date to early October, provided that cold-resistant

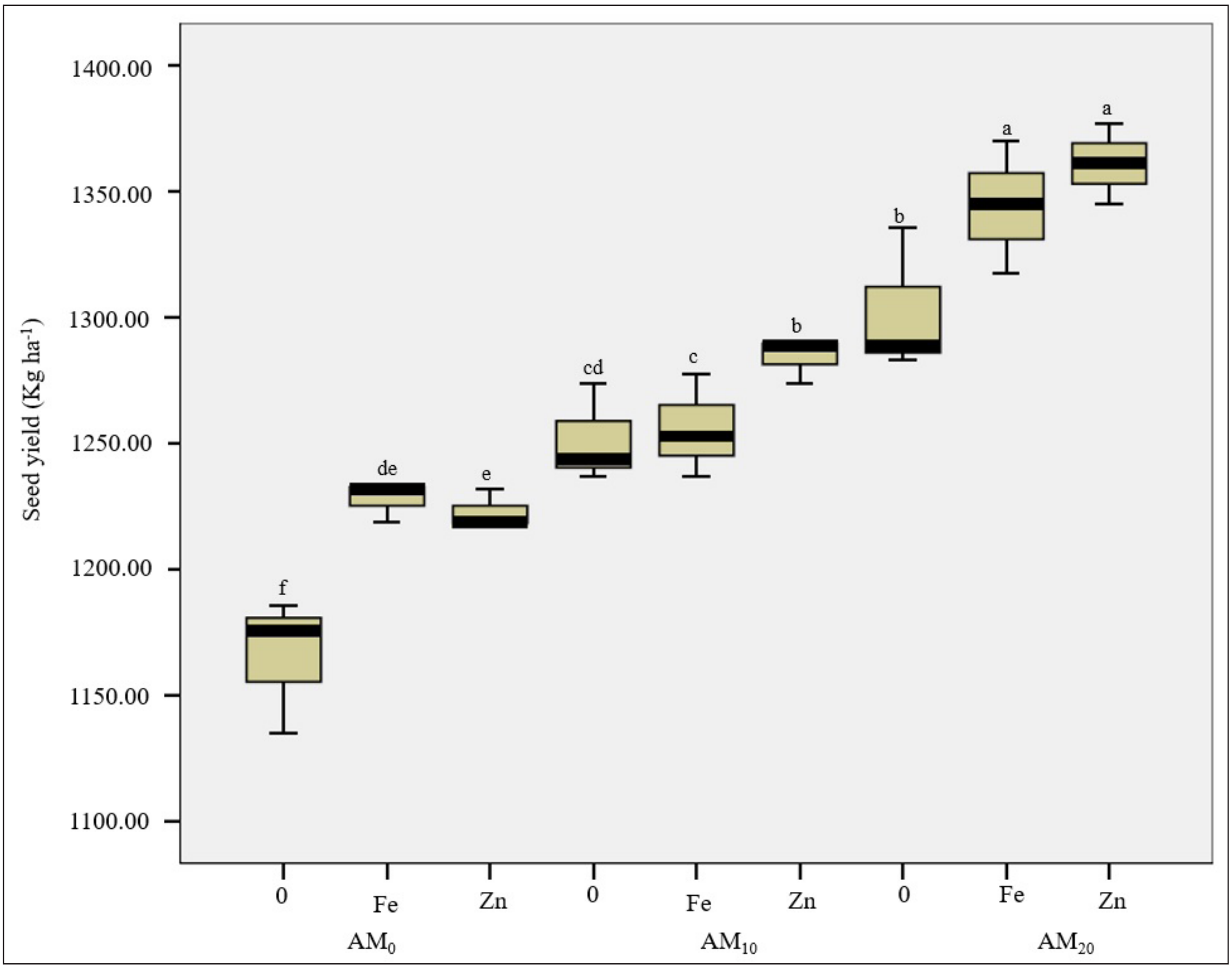

Fig. 3. Evaluation of the application of different levels of animal manure and rare elements on the seed yield of Camelina grown in the Damaq region in western Iran. AM_0, AM_{10}, and AM_{20}: application of 0, 10, and 20 t ha^{-1} animal manure, control (0) for trace element: no use of trace element fertilizer, Zn, and -Fe: the soil application of EDTA-chelated zinc or iron fertilizers during seed planting.

varieties are available, can significantly improve water use efficiency.

The findings of this study showed that with increased use of organic soil amendments (animal fertilizers), most vegetative traits and yield components improved. A study of the chemical and physical characteristics of the soil in the region indicated that nutrient availability and inappropriate characteristics such as low organic matter could be important factors limiting the yield of *Camelina sativa*. In such circumstances, organic soil amendment through the use of abundant, accessible, and affordable organic matter such as animal manures could be considered an ideal option. With increased use of animal manure, the initial stages of seedling growth were reduced by about 50% compared to the control. This stage is one of the most key stages for seedling establishment and ultimately determines the rate of water and light utilization. However, it is also indirectly important in improving competition with weeds. Accelerating seedling establishment can lead to timely root and shoot development and at the same time, the seedling can make optimal use of early season rainfall, ultimately increasing water use efficiency. Zinc application also improved the speed of seedling emergence compared to iron, which probably indicates a severe zinc deficiency in the soil or the effect of zinc on early seedling growth.

The use of high levels of organic soil amendments (AM_{20}) was able to increase both longitudinal and transverse canopy growth. This could be due to the direct supply of nutrients or improved chemical conditions to increase optimal nutrient uptake. It could also be attributed to the improvement of physical

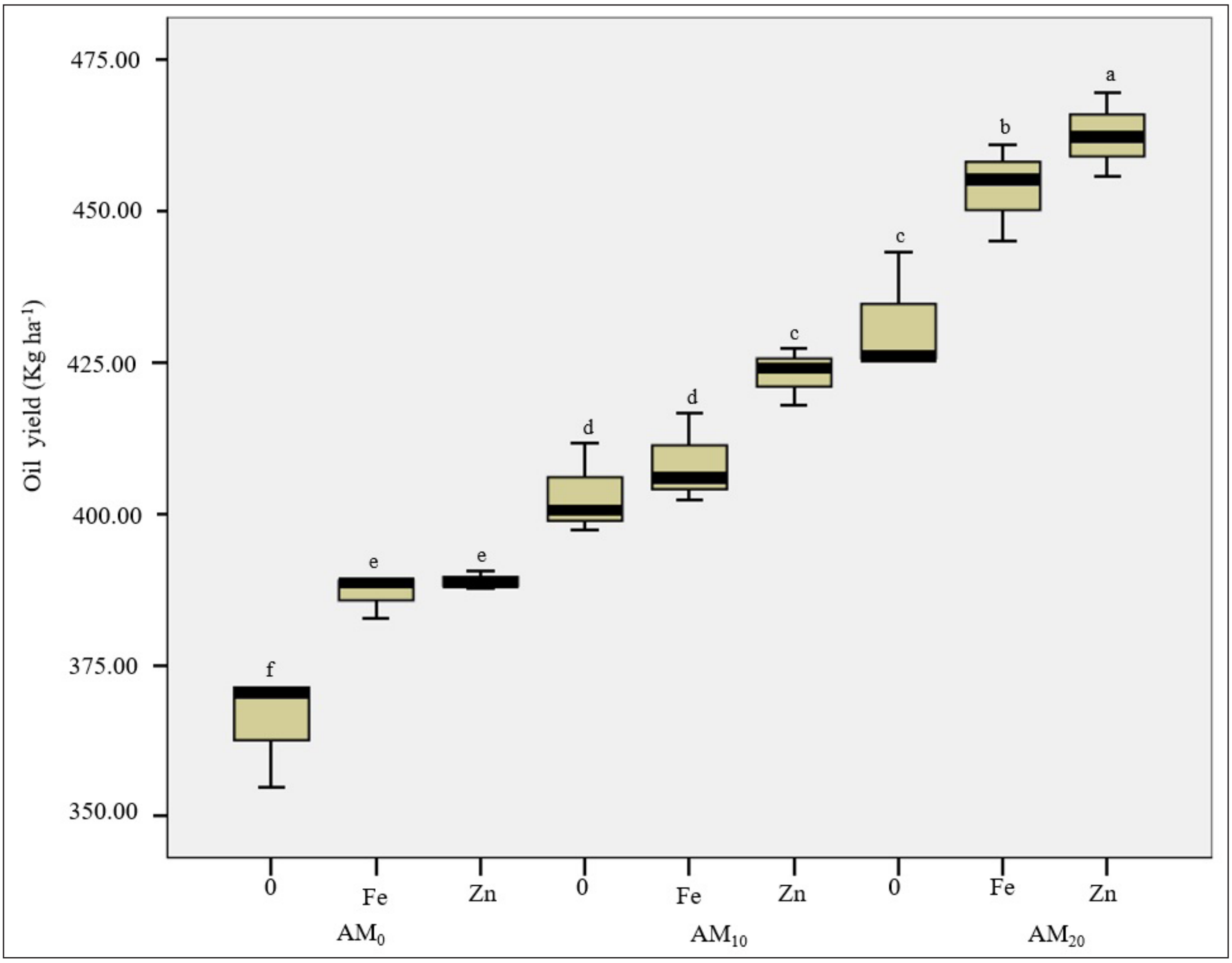

Fig. 4. The effect of animal manure and trace elements application on oil yield of camelina canopy in the semi-arid region of Damaq in western Iran. Columns with different letters are statistically significant at the 5% level. AM_0, AM_{10}, and AM_{20}: application of 0, 10, and 20 t ha^{-1} animal manure, control (0) for trace element: no use of trace element fertilizer, Zn, and -Fe: the soil application of EDTA-chelated zinc or iron fertilizers during seed planting.

properties and increased root growth in the soil, which improved water balance by increasing water uptake and supply to the aerial parts. Growth in plants is based on the accumulation of water in the vacuoles of cells, and increased water supply can provide increased growth by improving the turgor pressure. An important point in evaluating vegetative growth was that the effectiveness of trace elements improved with increasing use of animal manure. This indicates the existence of synergistic effects between animal manure and chemical fertilizer. While in AM_{10} conditions, the improving effect of iron on growth was greater than zinc, and with increasing use of animal manure, the effectiveness of zinc became much greater than iron. However, the effect of zinc on improving yield components and reproductive growth components in camelina was greater than that of iron. Number of siliquae per plant is considered the most important yield component. In addition to genetics, this component is also affected by environmental conditions. The formation of this component occurs at the end of vegetative growth and early reproductive growth. Under AM_0 conditions, the use of trace elements had no effect on the number of siliquae, but with the increase in the use of organic amendments in the soil, this yield component increased significantly. It seems that the use of organic amendments in the soil, by improving the chemical conditions of nutrient absorption and water retention capacity in the soil, has increased the functioning of photosynthetic apparatuses and improved resource capacity. By increasing the supply of photoassimilates, the ground has been prepared for the formation of a larger number of reproductive reservoirs or

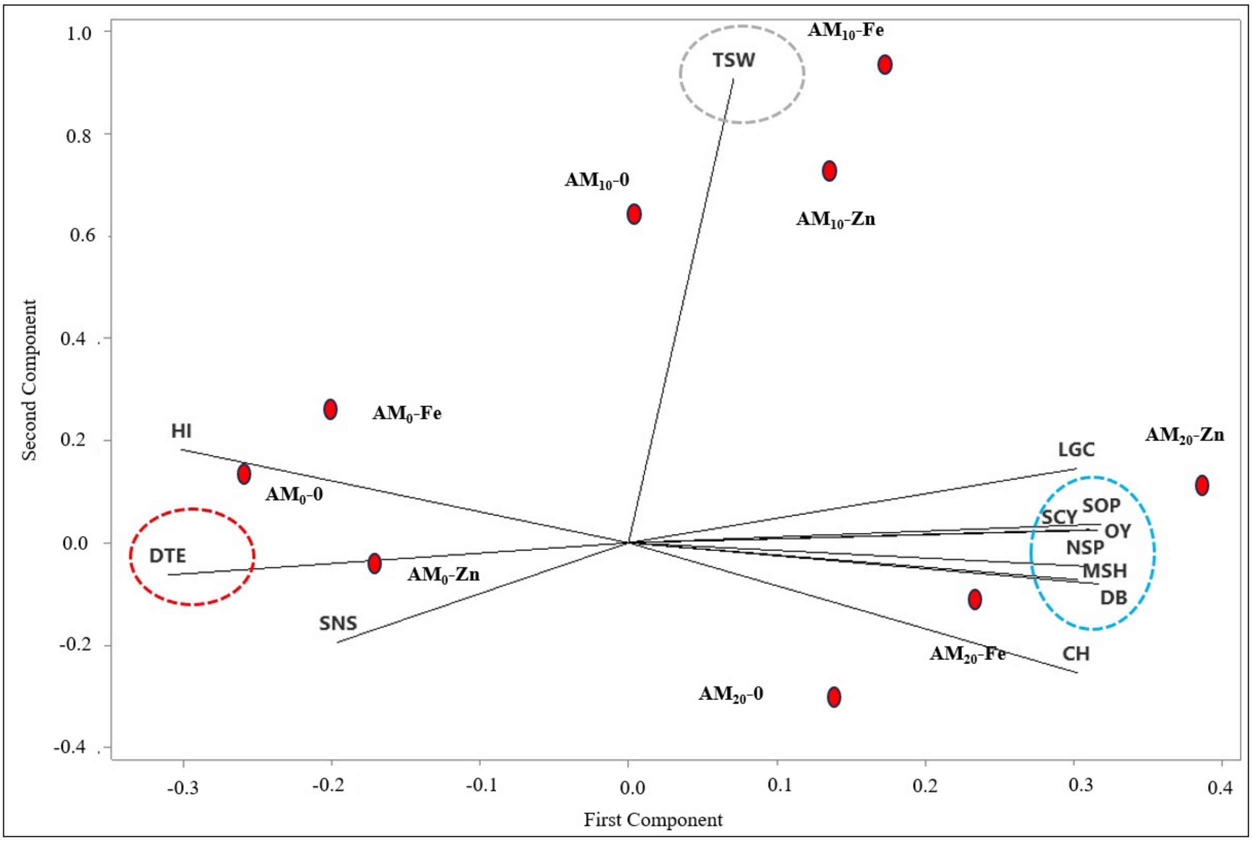

Fig. 5. Principal component analysis (PCA) plot to show the angular correlation between evaluated traits in Camelina sativa as well as the spatial location of combined treatments with different traits. DTE: day to seedling emergence, MSH: main stem height, LGC: lateral growth of canopy, CHI: chlorophyll content, NSP: number of siliquae per plant, SNS: seed number per siliquae, TSW: thousand seed weight, DB: dry biomass, SCY: Seed yield, HI: harvest index, SOP: seed oil percentage, OY: oil yield.

siliquae. This situation was evident even under irrigation deficit conditions. Hence, it can be stated that source-sink relationships are affected by the application of manures or trace elements. The results obtained showed that the combined use of organic amendments and trace elements increased the oil percentage in the seed by 2-3%. This, along with the increase in seed yield components, led to an increase in oil yield. The main purpose of camelina cultivation is oil production. In the aforementioned experiment, high levels of organic soil amendments along with zinc or iron provided the basis for the production of 450-470 kg ha^{-1} of oil even under deficit irrigation conditions.

It seems that organic fertilizers and micronutrients have provided the basis for increasing oil yield in *Camelina sativa* by increasing the speed of seedling establishment, increasing lateral canopy growth, increasing leaf area, improving chlorophyll content, increasing photosynthetic ability, increasing moisture available to roots, increasing the ability of the vascular system to transport photoassimilates, and improving the distribution and allocation of carbohydrates and compounds related to oil biosynthesis in the seed.

Conclusions

The results of this study showed that the use of animal manure in camelina production systems is an undeniable necessity. Considering the climatic conditions of the region, the use of animal manure was able to accelerate the speed of seedling emergence and establishment, and this is very effective in accelerating plant maturation and avoiding hot and dry periods at the end of the season. On the other hand, although the manure itself contained some micronutrients, the application of micronutrient fertilizers, especially zinc, improved the final performance compared to the control. The use of manure increased the effectiveness of micronutrient fertilizers. The application of iron along with high levels of manure improved vegetative growth characteristics and chlorophyll content. A compensatory relationship was observed between the yield components of Camelina. The highest seed yield and oil yield were obtained with the simultaneous application of 20 tons per hectare of manure + zinc. For further investigation in the future, it is recommended to consider chemical changes in the rhizosphere after the application of manure and micronutrients.

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