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The Impact of Planting Date, Foliar Iron and Zinc Nanofertilizer Application, and Ascorbic Acid on Fennel Yield, Yield Components, and Chlorophyll Content in a Climatic Environment (Case Study: Ilam Province)

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ABSTRACT

The purpose of this study was to examine how the climatic conditions of Ilam Province during the 2023 growing season affected the yield, yield components, and chlorophyll content of fennel (Foeniculum vulgare Mill.) in relation to planting date, foliar application of iron and zinc nanofertilizers, and ascorbic acid. Using a split factorial design based on a randomized complete block design with three replications, the experiment was conducted in two different climatic regions (Karzan, which is cold temperate, and Shabab, which is warm temperate). Planting dates (10, 20, and 30 Ordibehesht (Solar Hijri Calendar)), iron and zinc nanofertilizers (applied versus non-applied), and ascorbic acid at four concentrations (0, 4, 6, and 10 g/L) were all included in the experimental treatments. Grain yield, essential oil yield, number of umbels and umbellets per plant, number of seeds per umbellet, thousand-seed weight, and harvest index were all significantly decreased by delayed planting and higher temperatures. Later planting dates, however, resulted in a higher percentage of essential oil. The application of 6 mM ascorbic acid along with iron and zinc nanofertilizers at the earliest planting date produced the highest yield and quality indices. In addition to improving the leaves' relative water content, chlorophyll content, and leaf area index, ascorbic acid and nanofertilizer treatments also increased the activity of specific antioxidant enzymes, soluble sugars, and leaf protein content. Overall, applying iron and zinc nanofertilizers along with ascorbic acid at the ideal planting time can be regarded as a successful tactic for raising fennel production both quantitatively and qualitatively in Iran's varied climate.
Keywords: Fennel, Plant dates, Ascorbic acid, Chlorophyll, Nanofertilizer

INTRODUCTION

In the past few decades, medicinal plants have become increasingly important in the global food, cosmetic, and pharmaceutical industries. They have also helped to improve community health and support the growth of non-oil economies in a number of nations. Iran is a vital center for the production and genetic diversity of medicinal plants because of its exceptional biodiversity and distinct climate. International reports state that Iran is one of the top producers and cultivators of some species, including fennel (Foeniculum vulgare Mill) (Sultan Lu *et al*., 2015; Azimzadeh, 2009). Iran has a rich history of using medicinal plants in traditional medicine, which emphasizes the strategic significance of these resources in addition to its climatic advantages (Mozaffarian, 2009; Omidbeigi, 2008).

The active ingredients in fennel, which include anethole, fenchone, estragole, and limonene, make it a valuable industrial-medicinal plant that produces essential oils. It is used in traditional medicine to treat infantile colic, digestive issues, and to help nursing moms breastfeed more effectively. Fennel also serves as an aromatic and flavoring agent in the food and cosmetics industries (Singh *et al*., 2006; Omidbeigi, 2008; Hornak, 1992). According to global statistics, Iran is a major producer and exporter of fennel seeds, and its essential oil adds a significant amount of value to a variety of industries (Anonymous, 2012; Amir Teimuri *et al*., 2012).

Agronomic management techniques, temperature, planting date, soil properties, nutrient availability, and other environmental and genetic factors all have a significant impact on the chemical composition and quality of fennel's active compounds (Barros *et al*., 2010; Nguyen *et al*., 2015). The consistent production and quality of this valuable plant are severely hampered by biotic and abiotic stresses like drought and salinity, as well as by changes in the climate and water availability (Safaei *et al*., 2011; Rafii *et al*., 2007). Additionally, it is well known that secondary metabolites like flavonoids, antioxidants, and essential oils improve the stability and therapeutic efficacy of herbal products. Therefore, it is essential to investigate and create novel field management techniques to raise the amount and caliber of these compounds.

Many studies have shown that plant nutrition, especially concerning micronutrients (iron and zinc) and growth regulators such asascorbic acid, is essential for improving physiological processes and the synthesis of secondary metabolites, in addition to increasing growth and yield (Marschner, 2012; Vaziri Amjad, 2013). Enzymatic activity, chlorophyll synthesis, and the stabilization of cellular structure all depend on iron and zinc (Pandey *et al*., 2006; Ojeda-Barrios *et al*., 2014). Because of their increased absorption efficiency, enhanced nutrient bioavailability, and stimulation of physiological indices, the use of iron and zinc nanofertilizers has attracted attention (Nasiri *et al*., 2018). Furthermore, ascorbic acid is an important component of the plant's antioxidant system, which improves photosynthetic efficiency, stabilizes cell membranes, and increases tolerance to environmental stresses (Noctor & Foyer, 1998; Debolt *et al*., 2007).

Iron, zinc, and ascorbic acid applied separately improve growth characteristics, the quality of essential oils, and the production of metabolites, according to experimental research on a variety of medicinal plants (Akbarpour *et al*., 2021; Naghizadeh *et al*., 2022). However, there is still a lack of information about the combined and comparative effects of these treatments, especially for fennel in nano-formulations and under various climatic conditions. There are also significant unknowns about the effectiveness of combined strategies, the best application concentrations, and how these strategies interact.

The current study was designed in two different climatic regions of Ilam Province (Karzan and Shabab) to investigate the effects of planting date, foliar application of iron and zinc nano-fertilizers, and varying levels of ascorbic acid on fennel seed yield, yield components, and chlorophyll content in order to fill this scientific gap and provide a successful cultivation model for arid and semi-arid regions. The goal of the study is to determine the best combination of treatments to increase crop sustainability and productivity. The results of this study may be used to support the creation of novel management plans for the long-term production of therapeutic plants and to improve Iran's standing in the world market for fennel.

MATERIALS AND METHODS

Location and Experimental Conditions

The purpose of this study was to assess the effects of ascorbic acid treatments and iron and zinc nanofertilizers on the phenological, physiological, biochemical, and yield characteristics of the medicinal plant fennel (Foeniculum vulgare Mill) during the 2023–2024 cropping season. The experiment was conducted in two climatically different areas of Iran's Ilam Province: Shabab City (eastern region with warm temperate climate) and Karzan Village (northern region with cold temperate climate).

Karzan is 1,000 meters above sea level and has the geographic coordinates 33°48′ N latitude and 47°01′ E longitude. Shabab is 800 meters above sea level and has the coordinates 33°10′ N latitude and 46°20′ E longitude.

Prior to the experiment, composite soil samples were taken from each site using a random sampling technique from a depth of 0 to 30 cm in order to precisely describe the physical and chemical characteristics of the soil. In a dedicated soil laboratory, these samples were examined. Tables 1 and 3 list the characteristics of the soil, such as pH, electrical conductivity (EC), texture, organic matter content, total nitrogen, and available potassium and phosphorus.

The closest weather stations to each experimental site also provided meteorological data, such as mean soil temperature, annual precipitation, relative humidity, mean maximum and minimum air temperatures, and regional evapotranspiration during the cropping season (Tables 2 and 4).

Table 1. The physical and chemical properties of the soil at the first test site (Karzan)

pH	Electrical Conductivity (EC) (dS/m)	Soil Texture	Organic Matter (%)	Total Nitrogen (%)	Available Phosphorus (mppm)	Available Potassium (ppm)
81.7	91.0	Loam	29.10	14.0	07.11	218

Table 2. Meteorological information for the initial test location (Karzan)

Max. Temp. (°C)	Min. Temp. (°C)	Mean Temp. (°C)	Soil Surface Temp. (°C)	Mean Precipitation (mm)	Mean Minimum Humidity (%)	Mean Maximum Humidity (%)	Mean Evapotranspiration (mm/day)	Location
46.26	68.10	56.18	20.10	30.10	66.26	07.57	43.8	Karzan

Table 3. The physical and chemical properties of the soil at the second test site (Shabab)

pH	Electrical Conductivity (EC) (dS/m)	Soil Texture	Organic Matter (%)	Total Nitrogen (%)	Available Phosphorus (mppm)	Available Potassium (ppm)
12.70	95.0	Loam	3.00	3.00	05.80	300

Table 4. Meteorological information for the initial test location (Shabab)

Max. Temp. (°C)	Min. Temp. (°C)	Mean Temp. (°C)	Soil Surface Temp. (°C)	Mean Precipitation (mm)	Mean Minimum Humidity (%)	Mean Maximum Humidity (%)	Mean Evapotranspiration (mm/day)	Location
16.34	68.19	92.26	20.19	80.00	16.18	17.35	33.15	Shabab

Sampling and Trait Measurement

Phenological traits like "days to flowering" and "days to maturity" were sampled by means of ongoing monitoring and documentation during the plant's growth period. Established reference methods were used to measure physiological and biochemical traits: the amount of chlorophyll a and b was measured using the Porra *et al*. (1989) method, which involved sampling leaf discs, extracting them with diethyl ether, and measuring absorbance with a spectrophotometer at wavelengths of 663 and 645 nm. The absorbance at 470 nm was used to measure the carotenoid content. Wagner's method (Wagner, 1970) was used to measure the amount of anthocyanins in the leaves, and Irrigoyen *et al*. (1992) used the phenol-sulfuric acid method and absorbance at 485 nm to measure the amount of soluble sugar. A Delta-T leaf area meter and the associated formula were used to determine the leaf area index (LAI). Furthermore, fresh, turgid, and dry leaf weight measurements were used to calculate the leaf's relative water content (RWC) using the formula given by Mahmood *et al*. (2011).

Physiological and Biochemical Assessments

- Chlorophyll a and b: Porra *et al*. (1989) method: extraction with diethyl ether, absorbance using speci cformulas at 663 nm and 645 nm.
- Carotenoids: extracted simultaneously with chlorophyll, with absorbance measured at 470 nm.
- Wagner (1970) developed a method for anthocyanins with an extinction coefficient of 33,000.
- Using a Unico UV-230 spectrophotometer, the phenol-sulfuric acid method is used to measure soluble sugars.
- Soluble Proteins: Describe the solutions in detail if you are using the Bradford method.
- A Delta-T device is used to measure the Leaf Area Index (LAI), which is evaluated at each significant growth stage.
- According to Mahmoud *et al*. (2003), RWC, or relative water content, is

$$RWC = \frac{FW - DW}{TW - DW} \times 100 \qquad (1)$$

- FW: Fresh weight, DW: Dry weight, TW: Turgid weight.
- Electrolyte Leakage: According to the Blum standard.

Evaluation of Yield and Yield Component

Each plot's plants were manually harvested when they reached full maturity. Using exact weighing and counting techniques, yield components such as the number of umbels per plant, the number of umbellets per umbel, the number of seeds per umbellet, the thousand-seed weight, and the seed yield were ascertained. The ratio of seed weight to the total dry weight of above-ground biomass was used to compute the harvest index. The final results' treatment comparisons were based on these measurements.

Analysis of Essential Oil Content and Composition

A Clevenger-type device was used to hydro-distill dried fennel seeds in order to ascertain their essential oil content. A weight percentage (grams of oil per 100 grams of dry matter) was used to represent the essential oil yield. Gas chromatography (GC) was used to identify the constituents of essential oils by comparing retention times to established standards. Anethole, estragole, and fenchone were the main components found.

Experimental Design

In order to assess the individual and combined effects of different treatments, the experiment was carried out using a split-factorial design within a randomized complete block design (RCBD) with three replications. Planting dates, which were set at three levels (10, 20, and 30 Ordibehesht (Solar Hijri Calendar)), were the primary determinant. Foliar application of 12% chelated iron and zinc nanofertilizer, applied at two levels (no application and application), was the sub-factor. Foliar application of ascorbic acid, measured at four concentrations (0, 4, 6, and 10 millimolar), was the sub-sub-factor.

The dimensions of each sub-plot were 2 × 4 square meters, with 50 cm between rows, 15 cm between plants within rows, 1 m between plots, and 2 m between blocks. The commercial fennel cultivar seeds that were used were obtained from Iran Seed Company and went through quality control.

All agronomic tasks (land preparation, planting, weeding, irrigation, and harvesting) were carried out consistently in two areas with the same conditions in order to increase precision. A computer-generated randomized layout was used to determine the distribution of treatments in each region.

RESULTS AND DISCUSSION

The Shapiro-Wilk or Kolmogorov-Smirnov tests in SPSS software (version 16.0) were used to evaluate the normality of the data distribution for each trait and experimental errors prior to statistical analysis. Bartlett's test was used to assess homogeneity of variances for data gathered from various locations in order to ascertain whether combined analysis was feasible. Data from both sites were combined and examined after variance homogeneity was confirmed.

SAS software (version 9.4) was used to conduct analysis of variance (ANOVA) based on the split-factorial design within a randomized complete block design (RCBD). Ascorbic acid, foliar application of iron and zinc nano-fertilizer, and planting date were assessed for their individual and combined effects on all traits under study (Steel *et al*., 1997). At a 5% significance level, mean comparisons between treatment levels were performed using Duncan's Multiple Range Test. The results' significance levels were indicated using standard symbols and reported by APA guidelines.

Outliers were found and, if required, eliminated before the final analysis. ANOVA tables and mean comparison tables with standard deviations were used to display all of the results. To make it easier to interpret the results, Microsoft Excel was used to create graphs and other visual representations.

Preparation of Chitosan Solution

A precise 1 gram of chitosan powder (0.1% w/v; Sigma-Aldrich) was dissolved in 1000 milliliters of warm distilled water to produce the chitosan solution. 1% (v/v) acetic acid was added gradually dropwise while stirring constantly until the chitosan particles completely dissolved and a completely transparent solution was obtained (Hosseini *et al*., 2011). A potentiometer was used to bring the solution's pH down to about 5.6. To avoid its chemical structure degrading, the solution was established fresh for every application and used right away.

An 8-liter backpack sprayer with a conical nozzle was used for foliar spraying in order to guarantee even droplet distribution. To prevent cross-contamination, deionized water and meticulously cleaned equipment were used for each treatment. To optimize absorption efficiency, spraying was done early in the morning. The volume, concentration, and number of replications were meticulously standardized throughout all plots to guarantee treatment uniformity.

Yield and Yield Component Measurement

After the growing season, each plot's whole harvested area was manually harvested in order to calculate grain yield. Grain yield was computed as grams per square meter after the samples were weighed and dried at 25°C. The following parameter was one of the yield components that were assessed using standard protocols:

The quantity of umbels in each plant

Five randomly chosen whole plants from each plot were sampled at the end of the growth stage. Each plant's total umbel production was meticulously tallied, and the average number of umbels per plot was reported. This characteristic is essential for the plant's ability to reproduce as well as how responsive it is to treatments and environmental factors.

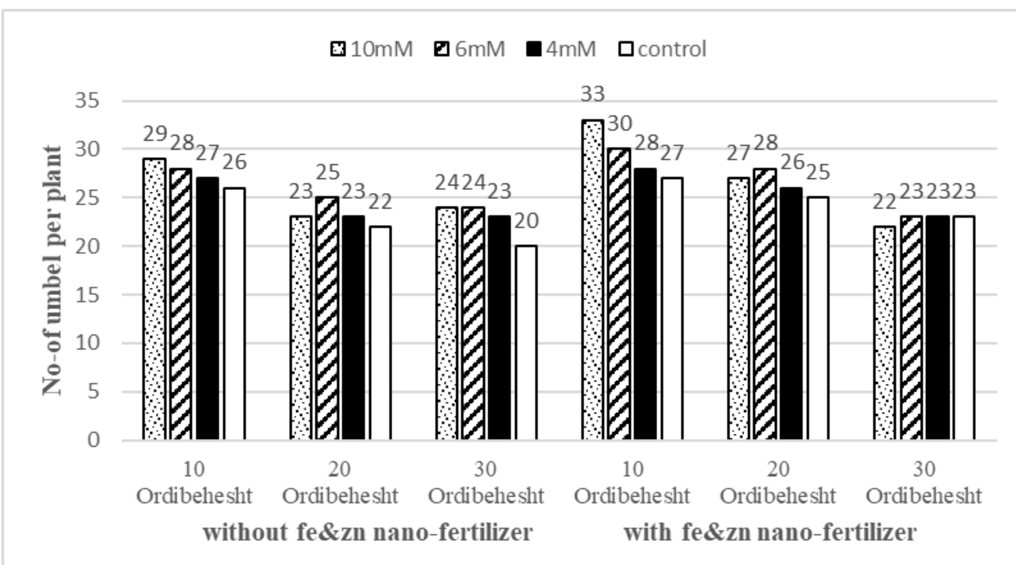

Figure 1. Average Comparison of the Triple Interaction Impact of Ascorbic Acid, Iron and Zinc Nano-Fertilizer, and Planting Date on the Number of Umbels per Fennel Plant

The number of umbellets in each umbel

Five primary umbels were chosen from each of the five plants that were sampled at the start of full flowering, and the number of umbellets in each umbel was carefully counted. Agronomic management, fertilizer application, planting date, light intensity, and temperature all affect this trait, which represents the inflorescence's ability to branch.

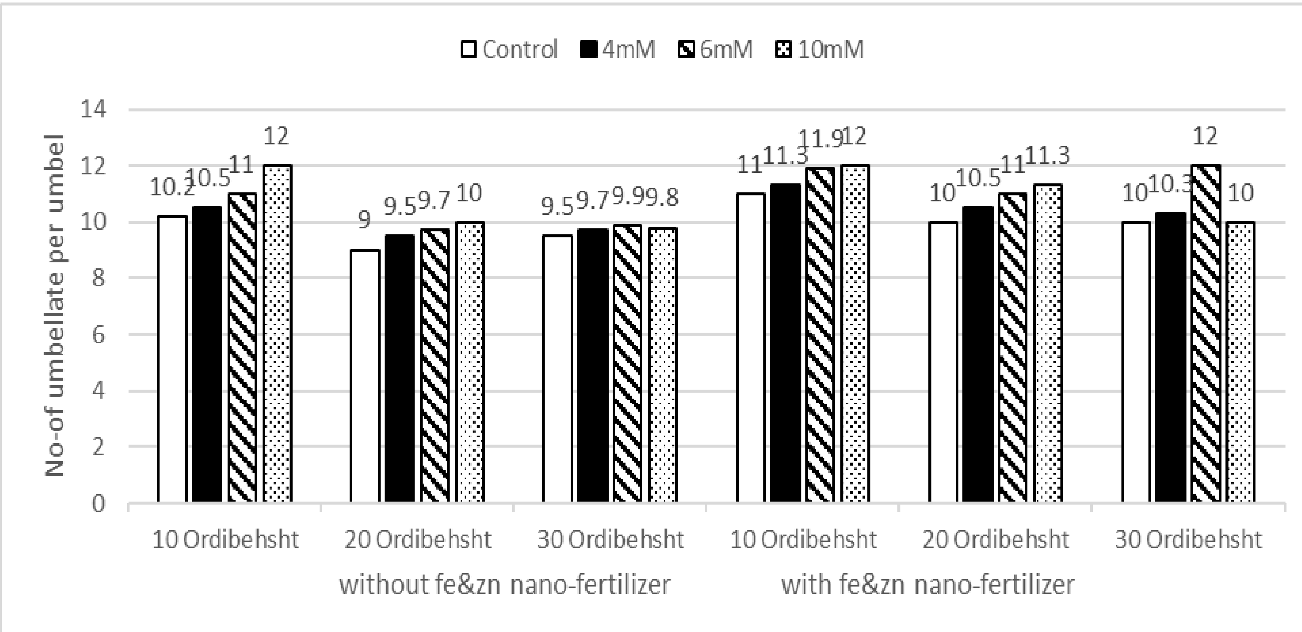

Figure 2. An investigation at the average three-way interaction effects of planting date, nano iron and zinc fertilizers, and ascorbic acid on the number of umbellets per umbel in fennel.

The number of seeds per umbellet

All of the healthy seeds in each chosen umbellet were meticulously counted after seed formation. The effectiveness of fertilization, pollination, and photosynthetic resource management are all reflected in this metric.

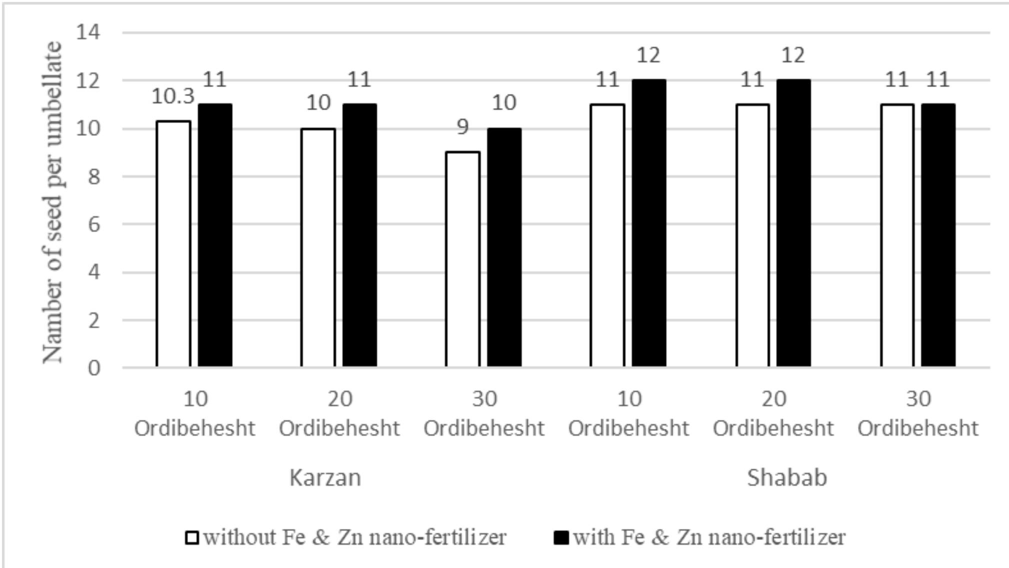

Figure 3. Interaction effects of planting date, nano iron and zinc fertilizers, and ascorbic acid on the number of seeds per umbellet in fennel.

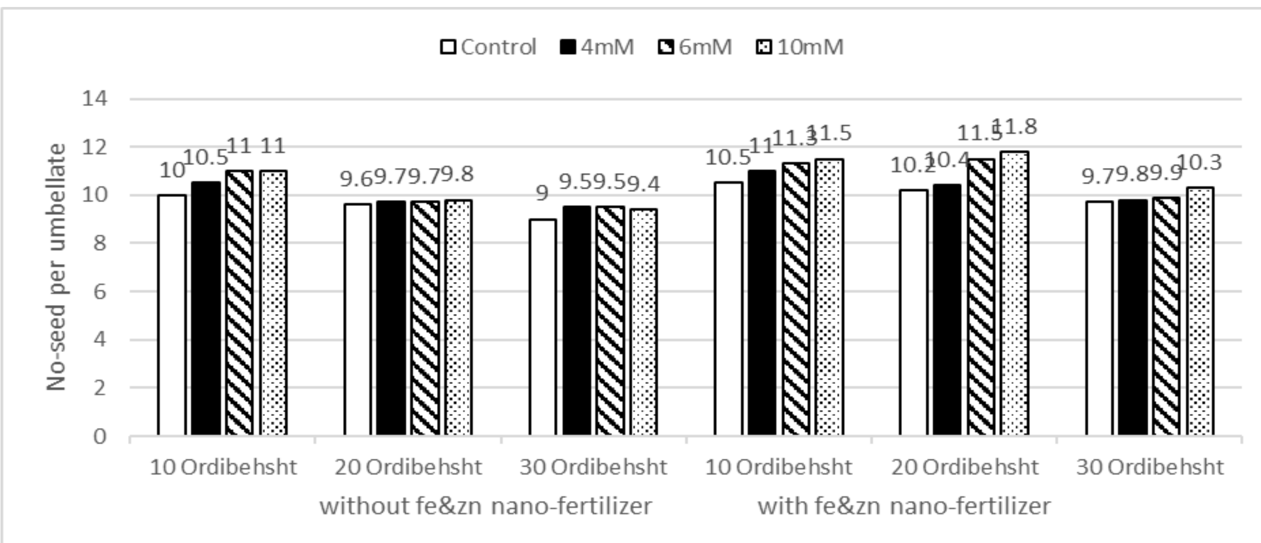

Figure 4. Interaction effects of location, planting date, and nano iron and zinc fertilizers on the number of seeds per umbellet in fennel.

Weight of a Thousand Seeds

Following harvest, 1,000 seeds were randomly selected from each experimental plot and weighed with a 0.0001 gram precision on a digital scale. Grain filling, seed health, and the results of stress-reduction and nutritional interventions are all indicated by this metric.

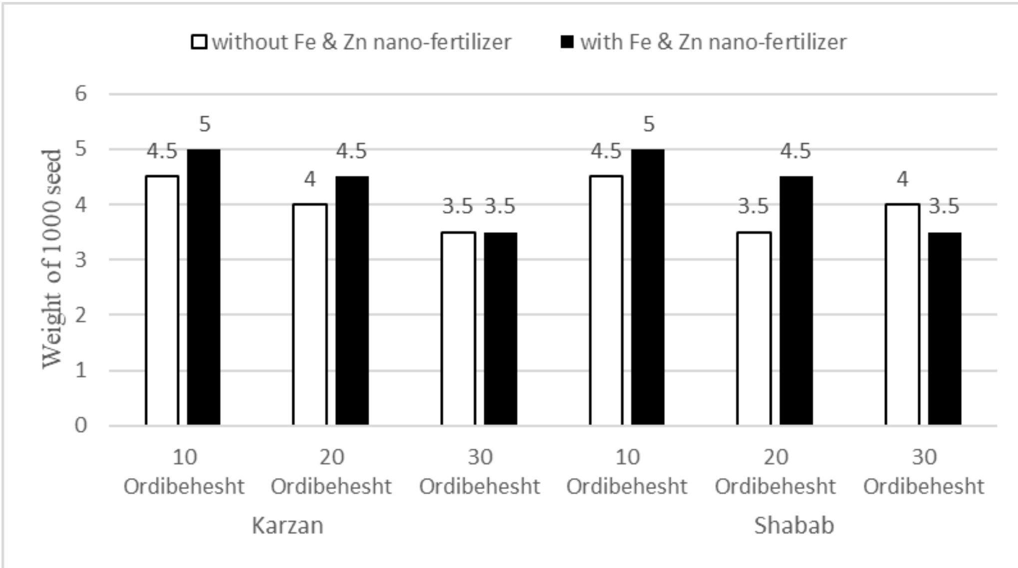

Figure 5. Comparison of the mean three-way interaction effects of location, planting date, and nano iron and zinc fertilizers on thousand-seed weight.

Grain Yield

Using a standard harvested area of 8 square meters, grain yield was calculated as the total weight of dry seeds harvested from each plot. This weight was then expressed in kilograms per hectare (kg/ha) or grams per square meter (g/m^2). When required, the ratio of seed weight to total aboveground dry biomass was used to compute and record the Harvest Index (HI).

Total Chlorophyll a, b, and a+b Content

Leaf Chlorophyll Extraction Method

Five healthy, randomly chosen plants were sampled at the 4–6 leaf stage, and the middle leaves were gathered. From each leaf, leaf discs measuring 0.5 cm in diameter were made. After being moved into sterile tubes with distilled water and polyethylene glycol, the samples were allowed to sit at room temperature for a full day before being dried with filter paper. Five milliliters of 90% diethyl ether were used to extract the chlorophyll. To guarantee complete extraction, the samples were stored at room temperature in the dark for five days (Porra *et al*., 1989).

A precision spectrophotometer (model UV-230) was used to measure the absorbance of the chlorophyll solution at wavelengths of 663 nm and 645 nm. The following formulas were used to determine the chlorophyll a and b concentrations (in milligrams per gram of fresh weight):

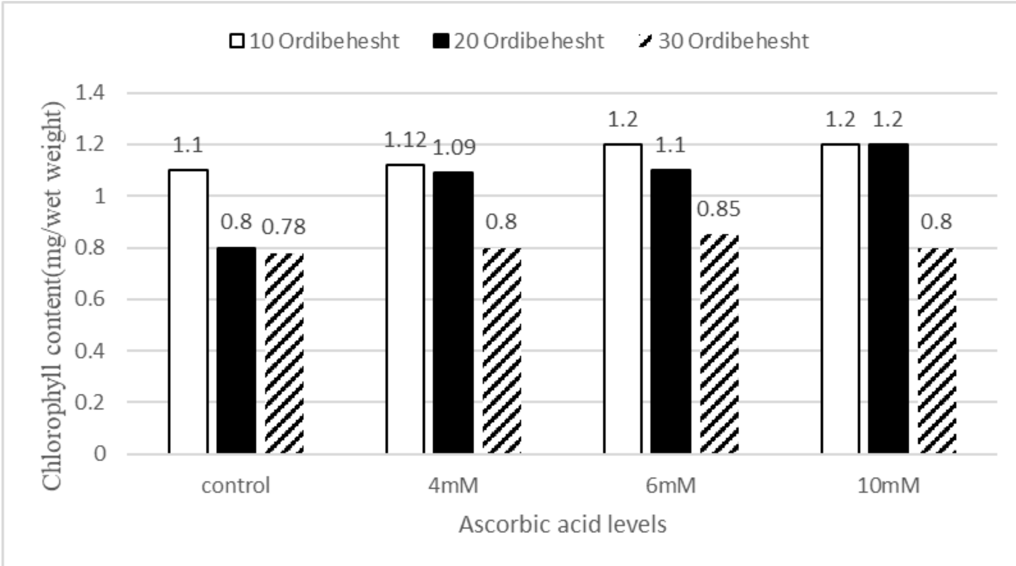

Figure 6. Mean Comparison of the Interaction Effect of Planting Date and Ascorbic Acid Levels on Chlorophyll a Content.

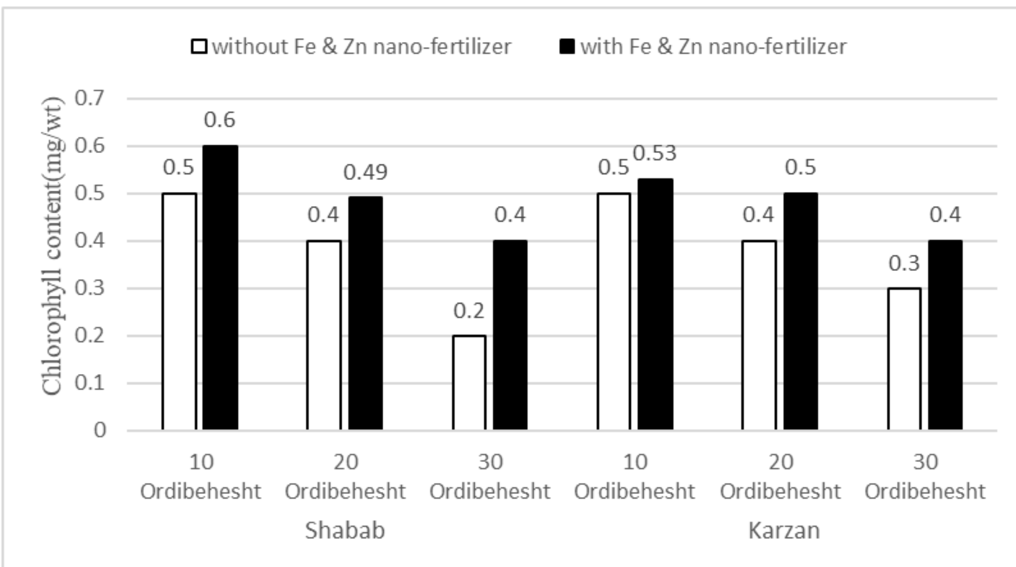

Figure 7. Mean Comparison of the Triple Interaction Effect of Location, Planting Date, and Iron and Zinc Nano-Fertilizer Levels on Chlorophyll b Content.

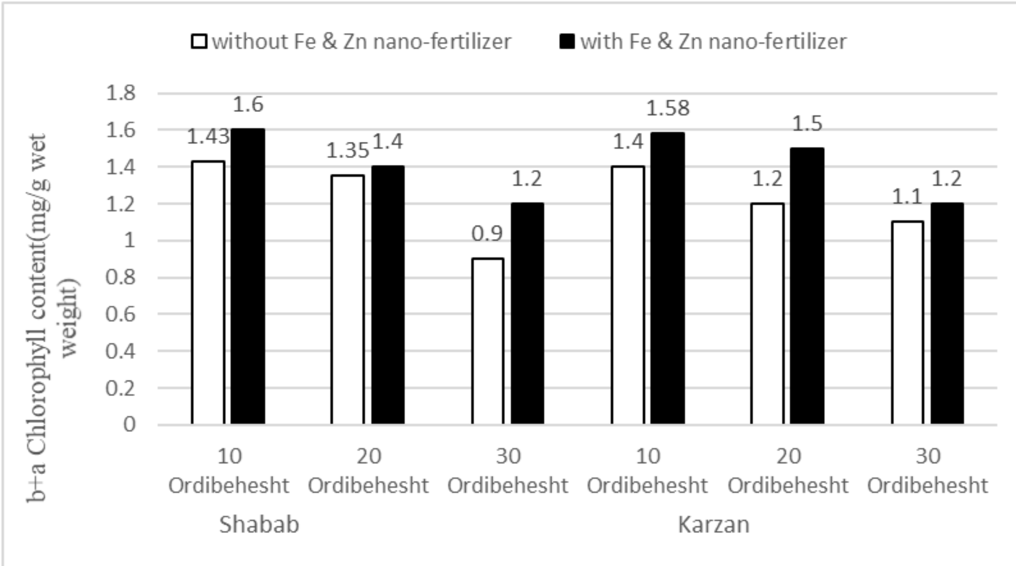

Figure 8. Mean Comparison of the Triple Interaction Effect of Location, Planting Date, and Iron and Zinc Nano-Fertilizer Levels on Total Chlorophyll (a+b) Content.

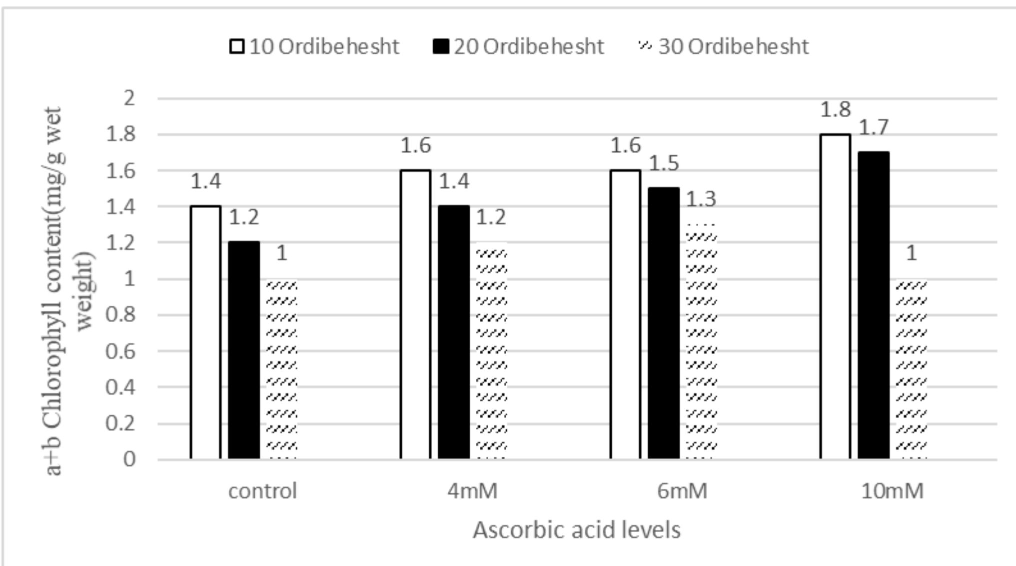

Figure 9. Mean Comparison of the Interaction Effect of Planting Date and Ascorbic Acid Levels on Total Chlorophyll (a+b) Content.

$$Chorophylla = (19.3 \times A_{663} - 0.86 \times A_{645}) \times V/(300 \times W)$$

$$Chorophylla = (19.3 \times A_{645} - 3.6 \times A_{663}) \times V/(300 \times W)$$

Where V is the extract's volume (mL), A is its absorbance, and W is the sample's fresh weight (g).

By adding the values of chlorophyll a and chlorophyll b, the total chlorophyll content (a + b) was determined.

DISCUSSION

The findings of this study unequivocally show that improving the quantitative and qualitative characteristics of fennel under various climatic conditions requires optimal nutritional management utilizing iron and zinc nanofertilizers in conjunction with ascorbic acid. Given that late planting dates were linked to higher temperatures, the observed decrease in seed yield and its constituent parts highlights how sensitive this medicinal plant is to heat stress, especially during crucial growth stages like flowering and seed filling. These results are consistent with earlier studies by Miraj *et al*. (2018) and Kandil *et al*. (2013), which showed that one of the most important factors in stabilizing yield and enhancing its constituent parts is careful fertilizer management and suitable planting dates.

The combined application of iron and zinc nano-fertilizers and ascorbic acid resulted in a notable increase in thousand-seed weight, seed yield, harvest index, and essential oil quality traits (anethole and estragole). This underscores the critical role that antioxidants and micronutrient nutrition play in improving plant efficiency and reducing oxidative stress (Sharif *et al*., 2018). Applying nano-fertilizers improved photosynthetic efficiency, translocation of photosynthates to seeds, and chlorophyll synthesis, which in turn increased yield and thousand-seed weight. Hosseini *et al*. (2021) highlighted the significance of iron and zinc in controlling enzymatic and physiological processes in plants, and these findings support the usefulness of ascorbic acid as a strong antioxidant in abiotic stress-prone environments.

The beneficial effects of the treatments on physiological characteristics, such as total chlorophyll content, chlorophyll a, and chlorophyll b, which support photosynthetic status, seed filling, and seed quality, are another noteworthy feature of this study (Porra *et al*., 1989). In the absence of ascorbic acid and nanofertilizers, the decrease in chlorophyll content and leaf area index during late planting dates supports the idea that environmental stressors inhibit important growth processes. A scientific foundation for the improvement of yield-related traits in the medicinal plant fennel is provided by the application of these treatments, which increase the amount of chlorophyll, stabilize photosynthetic activity, and increase the relative leaf water content.

Additionally, ascorbic acid and nanofertilizers work together to increase plant resistance to drought stress, as shown by decreased electrolyte leakage and enhanced antioxidant status. This leads research to develop technologies that are tailored to the semi-arid climate of western Iran. The development of sustainable agronomic strategies must take into account the fact that a plant's physiological and genetic traits, as well as intricate interactions with environmental factors, determine how it responds to different treatment levels.

The success of nutritional-hormonal management in reducing the negative effects of heat and moisture stress is demonstrated by the overall positive correlation between increased chlorophyll content and yield components, especially thousand-seed weight and total yield, as an indicator of photosynthetic potential. This emphasizes how important it is to use this combination of treatments in arid and semi-arid areas. The study's conclusions serve as a basis for technical advice and the enhancement of fennel production in regions with a moderate to low potential yield.

CONCLUSION

The results of this study clearly show that ascorbic acid and iron and zinc nanofertilizers, when combined with intelligent nutritional management, are essential for improving the medicinal plant fennel's quantitative and qualitative performance under a variety of climatic and environmental stressors. Nutritional treatments, especially the use of iron and zinc nano-fertilizers and ascorbic acid (at a concentration of 6 mM) together during early planting dates, improved physiological parameters like photosynthetic activity, chlorophyll content, and leaf relative water content, and greatly increased thousand-seed weight, seed yield, and harvest index. By significantly raising the percentage of important medicinal compounds like anethole and estragole, these treatments also improved the quality of fennel essential oil, highlighting the vital role that targeted nutrition plays in the industrial and pharmaceutical production of this plant. Additionally, the findings show that the negative effects of heat stress and water resource constraints can be significantly reduced by choosing suitable planting dates and applying micronutrients on time. In order to increase yield, improve stress tolerance, improve product quality, and ensure the sustainability of fennel production in areas with high risks of climatic variability, iron and zinc nanofertilizers and ascorbic acid can be applied simultaneously.

In order to guarantee timely planting dates, optimal utilization of micronutrients, and the application of antioxidants like ascorbic acid, farmers and producers of medicinal plants in semi-arid and arid regions are advised to modify their cultivation programs. Prioritizing this strategy will increase economic, qualitative, and quantitative productivity. In addition to improving the production system's sustainability, this approach raises fennel's added value and competitiveness in both domestic and foreign markets.

Future research should concentrate on figuring out when input applications should be made, examining the long-term impacts on other physiological characteristics and product quality, and performing cost-benefit analyses of the suggested therapies. These initiatives will open the door for the nation to grow fennel and other therapeutic plants in a sustainable and profitable manner.

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