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Effects of Different Seeding Rates on Growth Performance, Yield, and Quality of *Calendula officinalis* L. in Mediterranean Conditions

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ABSTRACT

"*Calendula officinalis* L., commonly known as pot marigold and a member of the *Asteraceae* family, is widely used in cosmetics, medicine, and pharmacy due to its rich bioactive compounds. This study investigated the effects of varied seeding rates on the growth, yield, and quality of *Calendula officinalis* L. in Mediterranean climates, focusing on morphological, quality, and agronomic characteristics, including seed and biological yield. The research was conducted at the experimental fields of the Ege University, Faculty of Agriculture, Department of Field Crops, over two growing seasons (2019–2020 and 2020–2021). The experiment followed a randomized complete block design with a factorial arrangement, incorporating three replicates. The factors included (a) year (2020 and 2021) and (b) five seeding rates (5, 10, 15, 25, and 35 kg ha^{-1}). Results indicated that seeding rate significantly impacted agronomic yield. While plant density per m^2 increased with higher seeding rates, there was a decline in plant branching, plant height, seed yield, biological yield, fresh flower yield, and dried flower yield at higher seeding rates. The optimum seeding rate for achieving high flower and seed yields was determined to be 10 kg ha^{-1}. Rainfall during the flowering period was found to be critical for maximizing drug flower and seed yield, highlighting the importance of careful consideration of seeding percentage and precipitation to achieve the optimum yield of *C. officinalis*.

1. INTRODUCTION

Calendula officinalis L., also known as pot marigold, is an annual or rarely perennial plant of the Asteraceae family. Native to the Mediterranean region, it is a medicinal and ornamental herb with yellow and orange flowers. It is a herbaceous (slightly woody at the base), 15-50 cm tall, multi-branched (Webb et al., 1988), usually secretory and aromatic plant (European Pharmacopoeia, 1975). Primarily, the flowers are used, but the stems, younger leaves, seeds and roots of *C. officinalis* L. also have medicinal properties. This versatile plant is used extensively the manufacture of pharmaceuticals (pain relief, calming the nervous system, promoting digestion, and treating skin diseases), cosmetics, culinary in medicinal, cosmetic, culinary and ornamental purposes (Warner and Erwin, 2005; Khalid and Teixeira da Silva, 2012; Ercetin et al., 2012). The essential oil of *Calendula officinalis* has been found to have various beneficial effects, including pain relief, calming nerves, promoting digestion, and treating skin diseases (Daniil and Kashchenko, 2013). Therefore, the essential oil and total flavonoid content of *C. officinalis* are significant factors that contribute to its pharmaceutical and medicinal importance.

The quantity and quality of crop products are significantly affected by cultural treatments like sowing date and seeding percentage, mineral fertilization, biotic stresses (diseases, pests and weeds), abiotic stress (harvest date, drying and storage conditions). These conditions significantly impact the production and concentration of secondary metabolites percentage. (Hussain et al., 2011; Berimavandi et al., 2011). Plants cannot fully utilize light, water and soil at extremely low densities. Similarly, intraspecific competition reduces yields at high densities (Seghatoleslami and Mousavi, 2009). Optimum plant density has been shown to provide more favorable conditions for crop development for higher flower yields (Karuppaiah and Krishna, 2005). Therefore, seeding rate is a critical element that impact on crop management components and yields. Low plant populations can diminish product by reducing seed yield and essential oil production (Panhwar et al., 2017).

The optimal seeding rate for crop production is influenced by several factors including soil moisture content and fertility, as well as the genotype or variety of the crop (Bukharov et al., 2023; Namdeo et al., 2020). The uniformity of seeding rate across the field is also important, as it affects the distribution of nutrition and moisture contents in the plants (Gupta, 2022). Additionally, economic factors such as the cost of seed and the price received from marginal yield increases can impact the optimal seeding rate (Lidsey et al., 2018). Other factors influencing the optimal yield are seeding percentage, planting date, soil type, drainage, soil fertility, pest pressure, and growing season conditions (Saberi et al., 2019). Precision agriculture techniques, such as variable seeding rate can help optimize seeding rate by taking into account field conditions and variability. Overall, achieving the optimal seeding rate requires considering a combination of biological, environmental, and economic factors.

Climate change poses additional challenges to seed production, including impacts on germination and plant development due to fluctuating temperatures and rainfall patterns (Maity et al., 2023; Bailly and Gomez Roldan, 2023; Moharana et al., 2023). Additionally, its can lead to changes in crop physiology and yield potential, with rising temperatures and weather extremes affecting (Roy and Rakshit, 2022). As climate change induces an upward shift in mean temperatures, the likelihood of supra-optimal conditions intensifies, potentially necessitating revisions in seeding rates to secure sufficient stand establishment (Cochrane, 2020).

The objective of study was to evaluate how different seeding rate treatments impact the productivity of plant flowers and seeds in response to varying climatic conditions in the Mediterranean region. This is an important area of research, as understanding the relationship between seeding rates and plant productivity can provide valuable insights for agricultural practices in regions with diverse Mediterranean climate.

2. MATERIAL AND METHODS

Climatic and soil characteristics

Izmir Province**,** located in the Mediterranean climate zone, is situated in the Aegean Region in the west of Türkiye, between the latitudes of 37° 45' and 39° 15' northern parallels and the longitudes of 26° 15' and 28° 20' eastern meridians. The study area was characterized by clay-loam soil (pH 7.88, and predominantly calcareous), with an organic matter content of 1.99%. The soil is rich in medium lime, with a lime content of 14.18%. Soil analysis indicated insufficient levels of organic matter, total nitrogen, and available phosphorus, but excessive available potassium in the field. According to long-term climatic data for Izmir province, the average maximum temperature is 27.0°C, the average minimum temperature is 3.9°C, the annual average temperature is 14.4°C, and the average yearly precipitation is 733 mm (Turkish State Meteorological Service, 2023). The climatic diagram of the experimental field for the years 2019, 2020, and 2021 is presented in Figure 1.

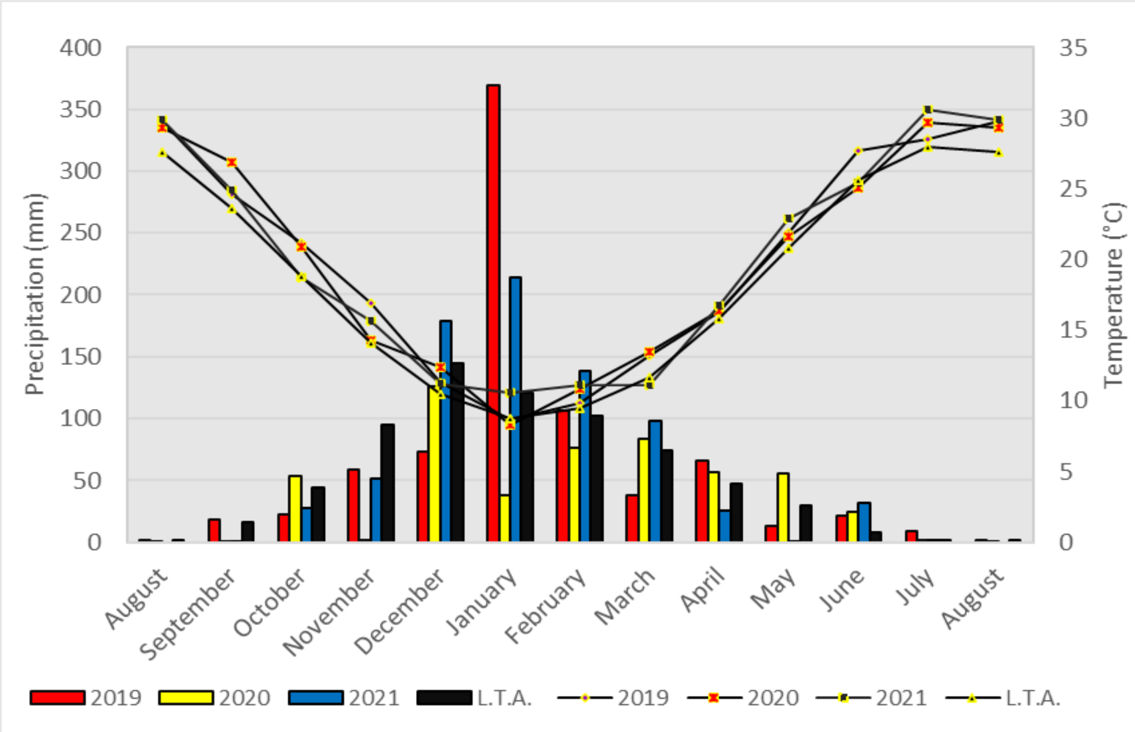

Figure 1. Precipitation and temperature of the experimental area.

In general, it is noteworthy that the temperature values observed in 2019, 2020, and 2021 were above the long-term averages (L.T.A.). For example, the temperatures in August were 29.8°C in 2019 and 29.9°C in 2021, compared to the long-term average of 27.6°C. This indicates a significant temperature increase in the summer months. Temperatures in January and February were also higher than the L.T.A. In particular, the temperature reached 10.6°C in January 2021, which is a significant increase compared to the long-term average of 8.8°C. There were considerable fluctuations in rainfall amounts. In particular, while 58.2 mm of precipitation was measured in November 2019, only 2.2 mm was recorded in the same month in 2020. This demonstrates an increase in the imbalance of the precipitation regime.

Additionally, significant increases in precipitation were observed in December. In 2021, December rainfall was 178.3 mm, well above the long-term average of 144.1 mm. Notably, 369.3 mm of rainfall was recorded in January 2019, nearly three times the long-term average of 121.0 mm (Fig. 1).

Plant material and field studies

The *C. officinalis* L. population seeds were used supplied by Pharmasaat GmbH (Germany) as research material. The field experiments were conducted on the trial area of the Field Crops Department, Faculty of Agriculture in Ege University in 2019-2020 and 2020-2021 growing seasons in Izmir-Turkey. The experiment followed a randomized complete block design with a factorial arrangement, incorporating three replicates. The factors included (a) year (2020 and 2021) and (b) five seeding rates (5 [SR1], 10 [SR2], 15 [SR3], 25 [SR4], 35 [SR5], kg ha^{-1}). Each plot consisted 4 rows, 40 cm apart and 3 m long. Plot sizes was 4.8 m^2, the total trial area was 96 m^2 in both the 2019-2020 and 2020-2021 growing seasons. The sowing dates were 8 November 2019 and 24 November 2020, in the same sequence. Seeding depth were 1-2 cm, and the seeds was covered using harrow. Sixty kilograms per hectare each of nitrogen and phosphate were applied to the seedbed. As basal fertilizer, phosphorus fertilizer TSP (triple super phosphate) was applied before sowing. Nitrogen (Urea) was given two times: as 30 kg ha^{-1} at the sowing stage, 30 kg ha^{-1} at the beginning of flowering period. During the experimental years, the plants were watered by a drip irrigation system. The weeds were controlled by hand and hoeing. No pesticides were used in used in the experiments.

Morphological and yield parameters

Ten plants were randomly sampled from each plot, and their morphological properties [(plant height (PH), total number of branches (TNB)]. Flower harvest, (the onset of flowering 12 April in 2020, 4 May in 2021) at the full bloom periods were measured at the time of harvest. Flower yield was determined by hand harvesting 1,6 m^2 area of the centre rows of each plots. The harvested plants whose fresh flowers yields (FFY) were calculated in each plot were left to dry in a drying cabinet at 30 ^{0}C for 2 days. The dry weights of the dried flowers were weighed on a precision balance and drug flowers yield (DFY) was determined in kg ha^{-1}.

Number of plant (NP), biological yield (BY), seed yield (SY) and harvest index (HI) were determined by collecting aboveground biomass. Plant samples were counted in the designated area using a 0.25 m^2 scale, and the number of plants per square meter was calculated. Seed yield was determined by hand threshing. The harvest index was determined by dividing the hand-threshed dry seed biomass by the total aboveground dry biomass.

Quality parameters

The essential oil rate (EOR) was measured by extraction from 10 g of air-dried (35 °C) drug material by hydro-distillation (100 mL water) with a Clevenger-type apparatus for a period of three hours (European Pharmacopoeia, 1975).

The total flavonoid content (TFC) was quantified using the aluminium chloride colorimetric assay. A solution of 5% $NaNO_2$ (0.3 mL) was added to the dry plant extract (0.3 mL), followed by the addition of 0.3 mL of 10% $AlCl_3$ solution after 5 minutes. Subsequently, 2 mL of 1 M NaOH was added, and the total volume was made up to 10 mL with distilled water. The solution was thoroughly mixed and incubated in the dark for 30 minutes. Absorbance was then measured spectrophotometrically at 510 nm. The total flavonoid content of plant samples was expressed as mg rutin per gram of dry matter. All spectrophotometric measurements were conducted using a Cary 50® spectrophotometer (Zhishen et al., 1999).

Statistical analysis

The statistical analyses were conducted using the JMP 14® statistical program (SAS Institute Inc., 2018). Statistically significant differences in seeding rate treatments were analyzed using a two-way analysis of variance (ANOVA) with the separation of means by a least significant difference (LSD) test ($p<0.05$). Grouped boxplots were created in R Studio via the ‘tidyverse’ (Wickham et al. 2019), ‘ggplot2’ (Wickham et al. 2016) and ‘gapminder’ (Bryan, 2022) packages. Additionally, correlations between all pairs of measured characters were calculated in R Studio.

3. RESULTS AND DISCUSSION

Morphological and yield parameters

Plant height (cm)

The year factor was statistically and significantly different ($p<0.05$). The maximum average plant height was 51.8 cm in 2021 and the minimum average was 43.9 cm in 2020 (Fig 2). The maximum plant height among the seeding rates was found using 10 kg ha^{-1} (48.8-54.8 cm) in both years, although the effect of seeding rate was not significantly different.

Rahmani et al. (2008) reported that drought stress in *C. officinalis* L. reduced plant height and caused smaller leaf area during the early vegetative growth period. Similarly, the decrease in plant height with drought stress was associated with an increase in the partitioning of assimilates between shoots and roots in agreement with Moosavi et al. (2014). Martin and Deo (1999) studied different seeding rates on flower production of *C. officinalis* L. in New Zealand. The study found the minimum plant height (54.4 cm) at 3 kg ha^{-1} seeding rate and the maximum (73.4 cm) at 48 kg ha^{-1}. Mollafilabi and Hosseini (2012) reported that the maximum plant height was found in 10 kg ha^{-1} seeding rates treatment and this value was found to be 26.4 cm. Mirzaei et al. (2016) determined the maximum plant height as 51.01 cm (60 plants m^{-2}). The researchers mentioned that the increase in plant height with increasing plant density can be explained by the increased activity of stem growth hormone due to lack of light. Kwiatkowski et al. (2020) came to the conclusion that an increase in plant height is associated with an increase in plant density, with the maximum height increase occurring at densities of up to 70-90 plants per m^2.

Plant height of *Calendula officinalis* showed a significant response to climate variability during the 2019-2020 and 2020-2021 growing seasons, particularly in terms of precipitation and temperature. Precipitation in the 2019-2020 season (November-May), in which the experiment was carried out, totaled 384.4 mm, while 603.1 mm was recorded in the same months of the 2020-2021 growing year. The results of the second year, the amount of precipitation effective in the period from sowing to flowering, increased approximately two times more than in the first year (Fig 1). The effects of the climatic conditions, which varied according to the year, and of increasing the seed rate up to a certain level on the plant height were significant ($p<0.05$). Increased rainfall in 2021 resulted in increased plant height, presumably due to increased water availability, facilitating cell elongation and overall growth. The increased height of the plants is beneficial for harvesting, but also makes them more susceptible to lodging in strong winds, especially if the height is excessive. Conversely, the reduced rainfall in 2020 resulted in less than optimal growth, highlighting the critical role of consistent water availability in maintaining healthy plant height.

Total number of branches (pcs plant^{-1})

The effect of seeding rates on total number of branches was found to be significant ($p<0.05$). The maximum value in total number of branches per plant was obtained from 10 kg ha^{-1} (11.7 pcs) and the minimum value was obtained from 35 kg ha^{-1} (5.67 pcs) seeding rate treatment. At low seeding rate, branching increased with the expansion of the unit area per plant. This may be attributed to the decrease in intraspecific competition with the decrease in the amount of seed per unit area. It is well established that the decrease in intraspecific competition results from lower seed density (Fig 2.).

The findings of study revealed a significant effect of seeding rates on the total number of branches (TNB) per plant ($p<0.05$), with the maximum TNB (11.7 branches) observed at a seeding rate of 10 kg ha^{-1} and the minimum (5.67 branches) at 35 kg ha^{-1}. These results suggest that lower seeding rates reduce intraspecific competition by expanding the unit area available per plant, thereby promoting branching. Our findings are consistent with the literature, where Martin and Deo (1999) established that increased plant density correlates with a decrease in branching and the harvest index. Similarly, Berimavandi et al. (2011) reported a TNB maximum of 9.44 branches per plant under conditions comparable to those in this study. Additionally, studies conducted in Türkiye, Bicen (2020) and Barut et al. (2022) reported TNB values ranging from 5.02 to 6.7 and 7.04 to 7.43 branches per plant, respectively. While these findings align with the trends observed in this research, variations may stem from differences in research methodologies and the influence of specific environmental conditions. Climatic variability during the study period also played a critical role in determining TNB. It is hypothesized that the reduced rainfall in 2020 may have restricted branching due to water stress affecting meristem activity. However, the higher and more variable rainfall in 2021 may have alleviated some of this stress, allowing plants to utilize available resources more effectively at optimal seeding rates.

Number of plants (plants m^{-2})

The interaction between year and seeding rate had a significant effect on the number of plants ($p<0.05$). Figure 2 illustrates that the maximum number of plants per square meter was observed in both 2020 and 2021 at a seeding rate of 35 kg ha^{-1}. Specifically, in 2020, this seeding rate resulted in a value of 98.7 plants m^{-2}, while in 2021, it yielded 82 plants m^{-2}. Conversely, the minimum number of plants was consistently recorded at a seeding rate of 5 kg ha^{-1} in both years. Upon considering the seeding rate concerning the average number of plants per year, it is evident that in 2021, the number of plants decreased compared to 2020. Nonetheless, the overall average number of plants across both years was calculated to be 41.4 plants m^{-2}.

The significant interaction between year and seeding rate on number of plant ($p<0.05$) observed in our study is consistent with findings in the literature, where plant density influences various growth parameters. The results showed that the maximum number of plants was recorded at a seeding rate of 35 kg ha^{-1}, with a decrease in 2021 compared to 2020. These findings align with studies like Cromack and Smith (1998), who noted that 40 plants m^{-2} or higher densities have minimal effects on crop growth and yield. Their findings suggest that increasing plant density does not significantly enhance the yield beyond a certain threshold, which may explain why a higher seeding rate (35 kg ha^{-1}) did not lead to a proportional increase in plant numbers in 2021. Similarly, Martin and Deo (1999) observed a decline in the number of flowers as plant density increased. This suggests that while the number of plants may rise with higher seeding rates, factors like competition for resources (light, water, nutrients) may negatively impact flower production, a key yield component. This may explain the observed differences in plant number and flower production in our study, as the seeding rates of 35 kg ha^{-1} did not result in the highest flower yields. Mollafilabi and Hosseini (2012) found that the maximum number of plants occurred at a seeding rate of 15 kg ha^{-1}, but higher densities did not significantly affect yield parameters. This observation further supports the concept that plant density may not always translate into higher yield, as other factors such as soil fertility, water availability, and plant spacing may play more critical roles in determining the final yield. Furthermore, the findings by Mirzaei et al. (2016) on dry flower yield in relation to plant density provide valuable insight into how plant density can influence not only the number of plants but also their biomass. They found a positive correlation between plant density and dry weight, with higher densities resulting in increased dry weight. This suggests that higher seeding rates might enhance certain aspects of growth (such as dry weight) even if the number of plants per square meter decreases, which could be a key consideration when optimizing seeding rates for flower yield. In conclusion, while higher seeding rates like 35 kg ha^{-1} lead to more plants per square meter in some years, the overall yield and quality depend on a complex interaction of factors, including year, density, and environmental conditions. The literature supports the idea that optimal plant density does not always equate to higher yields, and in some cases, there are diminishing returns as plant competition for resources increases.

Fresh flower yield (kg ha^{-1})

The statistical analysis results revealed a significant interaction between year and seeding rate on fresh flower yield ($p<0.05$). Notably, in 2021, the maximum fresh flower yield was observed at a seeding rate of 10 kg ha^{-1}, reaching 6.277 kg ha^{-1}, followed by a seeding rate of 5 kg ha^{-1}, yielding 6.088 kg ha^{-1}. Conversely, the minimum fresh flower yield occurred in 2020 at a seeding rate of 5 kg ha^{-1}, with a yield of 3.509 kg ha^{-1}. The average fresh flower yield across treatments was 4.186 kg ha^{-1} in 2020 and increased to 5.393 kg ha^{-1} in 2021. Over the study period, there was a notable 28.9% increase in the average fresh flower yield in 2021 compared to 2020. The average fresh flower yield across all seeding rate treatments was 4.789 kg ha^{-1}, with the maximum yield recorded at 10 kg ha^{-1} (5440 kg ha^{-1}). Remarkably, both years exhibited a decreasing trend in fresh flower yield beyond the 10 kg ha^{-1} seeding rate, with yields of 4.600 kg $ha^{-1}$and 6.277 kg ha^{-1} in and 2021, respectively (Fig 2.).

In 2019–2020, although rainfall was low but regular, it supported the water needs of *Calendula officinalis* L. during its growth stages, positively affecting fresh flower yield. However, insufficient water limits flower biomass and yield, especially during flowering. Higher seeding rates (e.g., 20 kg ha^{-1}) led to increased competition, which likely contributed to the reduction in yield. In 2020–2021, while rainfall was higher but irregular, the plants' growth was influenced by fluctuating water availability during flowering (Fig 1.). Despite this, the fresh flower yield was higher than in 2019–2020, particularly at lower seeding rates (e.g., 10 kg ha^{-1}), where plants showed improved flower biomass and yield (Fig 2.).

In the previous studies, Martin and Deo (1999) noted that lower plant densities increased flower weight per plant but decreased overall yield per hectare. Similarly, in our study, the highest yield was obtained at 10 kg ha^{-1}, while higher seeding rates beyond this point led to a decline in yield. This suggests that while plant weight might increase at higher densities, resource competition negatively impacts overall yield. Ganjali et al. (2010) and Mollafilabi and Hosseini (2012) also reported that increased plant density led to reduced flower yield. This supports the idea that higher seeding rates cause plant competition for resources like water, nutrients, and light, which ultimately lowers yield. In conclusion, the results of our study indicate that the relationship between seeding rate and flower yield is complex. The 10 kg ha^{-1} seeding rate optimizes fresh flower yield, while higher seeding rates lead to yield loss due to increased competition.

Drug flower yield (kg ha^{-1})

The statistical analysis unveiled a significant interaction between year and seeding rate concerning drug flower yield (p<0.05). The findings suggest that increasing the seeding rate positively influences drug flower yield each year. Specifically, in 2021, the maximum flower yield of 1.571 kg was attained with a seeding rate of 10 kg ha^{-1}. Likewise, in the same year, a seeding rate of 5 kg ha^{-1} resulted in a yield of 1.479 kg ha^{-1}, which statistically matched the yield obtained with the 10 kg ha^{-1} application. Conversely, the lowest drug flower yield was observed in 2020 with a seeding rate of 5 kg ha^{-1} (Fig 2.).

The findings are consistent with the broader literature but also reflect variations based on the experimental conditions. Martin and Deo (1999) identified optimal seeding rates ranging from 3 to 24 kg ha^{-1}, thereby establishing a foundational relationship between plant density and yield. However, Ganjali et al. (2010) and Mollafilabi and Hosseini (2012) reported no significant differences in drug flower yield under varying plant density treatments, suggesting that the effect of density may be context-dependent. Berimavandi et al. (2011) noted a maximum yield of 1.320 kg ha^{-1} at a density of 60 plants m^{-2}, while Mirzaei et al. (2016) observed a positive correlation between plant density and dry flower weight, achieving an exceptional yield of 211.19 kg ha^{-1} at similar densities. The differences observed in this study and previous research underscore the complex interplay of factors such as seeding rate, environmental conditions, and management practices. The high yields achieved in 2021, particularly at lower seeding rates, may be attributed to favorable climatic conditions, which mitigated resource competition and enhanced plant productivity. Conversely, the lower yields in 2020 highlight the adverse impact of water stress and variable precipitation on flower development. The findings emphasise the need for tailored agronomic practices, including precise seeding rate adjustments, to optimise drug flower yield under varying environmental conditions. Selecting an appropriate seeding rate, such as 10 kg ha^{-1}, coupled with effective water management, can ensure sustainable production while mitigating risks associated with climatic variability.

Seed yield (kg ha^{-1})

The effect of seeding rates has been found to be significant on seed yield (p<0.05). In trial years, the average seed yield values for seeding rate applications ranged from 1.020 to 1.724 kg ha^{-1}, with the peak yield occurring in 2020. The maximum yield was recorded in the 10 kg ha^{-1}seeding rate application (1.671 kg ha^{-1}), followed by 35 kg ha^{-1} (1.535 kg ha^{-1}) and 25 kg ha^{-1} (1.500 kg ha^{-1}) applications, respectively. Conversely, the minimum seed yield of 919 kg ha^{-1} was observed in the 5 kg ha^{-1} seeding rate application. However, the maximum seed yield per hectare was achieved in 2020, with 207 kg ha^{-1} from the 10 kg ha^{-1} seeding rate application, while the minimum yield was noted in 2021, with both 5 kg ha^{-1} and 15 kg ha^{-1} seeding rates (Fig 2.). Despite this variation, the interaction effect of year x seeding rates on seed yield did not reach statistical significance.

Seed yield varied between years, with a decrease in the second year's values compared to the first year. Climatic factors during the growing years were observed to limit seed yield, but the 10 kg ha^{-1} seeding rate consistently maximized seed yield, regardless of the year. A comparison of total precipitation from seed sowing to the last harvest date in the trial years (2019-2020 and 2020-2021) showed that the first trial year received 464.9 mm of precipitation, lower than the second year's 635.1 mm. Despite this, the first trial year exhibited more consistent rainfall during the plant's emergence and flowering period, while the second year had more fluctuation (Fig 1). The reduced rainfall, especially during the flowering period, is thought to limit plant productivity. Even with the decrease in average seed yield due to climatic factors, the 10 kg ha^{-1} seeding rate maximized seed yield in both years (Fig 2.).

The findings of Rahmani (2008), Jevdovic et al. (2013), and Telci et al. (2023) support the current study, highlighting the importance of irrigation and precipitation for seed yield in *C. officinalis* L.. Rahmani (2008) showed that delayed irrigation significantly reduces seed yield, which aligns with the reduced yields observed in the second year of this study due to fluctuating rainfall. Jevdovic et al. (2013) and Telci et al. (2023) emphasized the role of precipitation during the vegetative phase, which was less stable in the second year and may have hindered plant growth. Additionally, studies by Seghatoleslami and Mousavi (2009) and Mollafilabi and Hosseini (2012) suggest that high seeding rates lead to increased plant density, which can reduce seed yield by limiting factors like branch number and canopy cover. This finding is consistent with the current study, where the 10 kg ha^{-1} seeding rate produced the highest seed yield, emphasizing the importance of balanced plant density for optimal production. In the instance, with changing climatic conditions, it is important to carefully manage the of seeding rate and irrigation needed. The variability in yields, exacerbated by extreme meteorological phenomena, necessitates an increase in optimal seed production volumes, thereby affecting the financial requirements for seed producers (Serhatli et al., 2019). As the probability of extreme events escalates, anticipated profits diminish while the ideal planting volumes expand, thereby complicating supply chain logistics and market distribution (Serhatli et al., 2020).

Biological yield (kg ha^{-1})

The statistical analysis showed a significant interaction between seeding rate and year on biological yield (p<0.05). The maximum biological yield value was obtained from the 10 kg ha^{-1} seed application in 2020 (11.180 kg ha^{-1}). The minimum biological yield value was determined in the 15 kg ha^{-1} and 25 kg ha^{-1} seed treatments in 2020. Based on the results obtained for biological yield, variability was observed in the biological yield values for the different seed treatments during the trial years. In the first year, the biological yield ranged from 5.508 to 11.180 kg ha^{-1}, while in the second year it varied from 6.350 to 8.660 kg ha^{-1}. In particular, the maximum biological yield in 2020 was achieved with a seeding rate of 10 kg ha^{-1}, indicating that this specific seeding rate resulted in optimal yield performance in that year. Similarly, in 2021, the maximum biological yield was achieved at seeding rate of 35 kg ha^{-1} and 10 kg ha^{-1}, suggesting that this seeding rate was most conducive to maximizing yield in that year (Fig 2.).

The findings align with Martin and Deo (1999), who emphasized that seeding rates significantly influence biomass production, with higher yields observed at higher seeding rates. However, the observed variation in yield across different seeding rates in this study parallels the work of Seghatoleslami and Mousavi (2009), who found that optimal biological yields are not always achieved at the highest densities but depend on specific plant density thresholds. Mirzaei et al. (2016) reported increased plant dry weight with higher plant density, which appears partially consistent with the 2021 findings of this study, where a seeding rate of 35 kg ha^{-1} produced the maximum biological yield. Nevertheless, the results from 2020, where the 10 kg ha^{-1} seeding rate outperformed others, underscore the influence of environmental factors, such as precipitation patterns and temperature variations, on yield. The variability in biological yield between the two trial years highlights the role of climatic conditions (Fig 1.). In 2020, regular rainfall patterns during critical growth stages likely contributed to the superior yield at the 10 kg ha^{-1} seeding rate. Conversely, in 2021, despite higher total rainfall, its irregular distribution may have influenced the results, favoring a broader range of seeding rates, including the 35 kg ha^{-1} rate. This supports Jevdovic et al.'s (2013) findings that rainfall significantly affects biomass production during plant growth and flowering. Rahmani (2008) similarly emphasized the importance of water availability, showing that irrigation scheduling significantly impacts biomass production.

Harvest index (%)

The effects of the year x seeding rates interaction on the harvest index parameter were found statistically significant (p<0.05). The maximum harvest index value was determined at 25 kg ha^{-1} in the first year, followed by 35 kg ha^{-1} and 15 kg ha^{-1} seeding rate treatments. The minimum harvest index value was determined at 15 kg ha^{-1} seeding rate in the second year (Fig 2.). The harvest index reflects the ratio of seed yield to biological yield. In 2020, the higher HI values likely resulted from favorable climatic conditions that allowed for increased seed yield relative to biological yield. Conversely, the lower HI values in 2021 can be attributed to higher biological yields combined with a decline in seed yield. This result underscores the influence of environmental factors, particularly the irregular rainfall patterns observed in 2021, which likely limited seed production while promoting vegetative growth. In addition, it was determined that the harvest index values of the seed treatments in 2020 were twice as high as those in 2021, except for the 10 kg ha^{-1} seeding rate. It is noteworthy in this regard that the 10 kg ha^{-1} seed application yielded similar results in both years.

The findings of this study partially align with Martin and Deo (1999), who reported no statistically significant effect of seeding rates on HI but observed an optimal value at a seeding rate of 24 kg ha^{-1}. However, the negative correlation between increased plant density and HI observed in studies by Seghatoleslami and Mousavi (2009) and Mollafilabi and Hosseini (2012) was not fully supported here. While the HI decreased at higher seeding rates (e.g., 35 kg ha^{-1}) in 2021, in 2020, higher seeding rates such as 25 kg ha^{-1} and 35 kg ha^{-1} produced higher HI values. The consistent performance of the 10 kg ha^{-1} seeding rate across both trial years is noteworthy, as it suggests a robust adaptability to varying environmental conditions. This rate may provide a more stable harvest index compared to higher seeding rates, which were more sensitive to climatic fluctuations. In contrast, higher seeding rates exhibited greater variability in HI, potentially due to competition among plants for limited resources, particularly under suboptimal conditions.

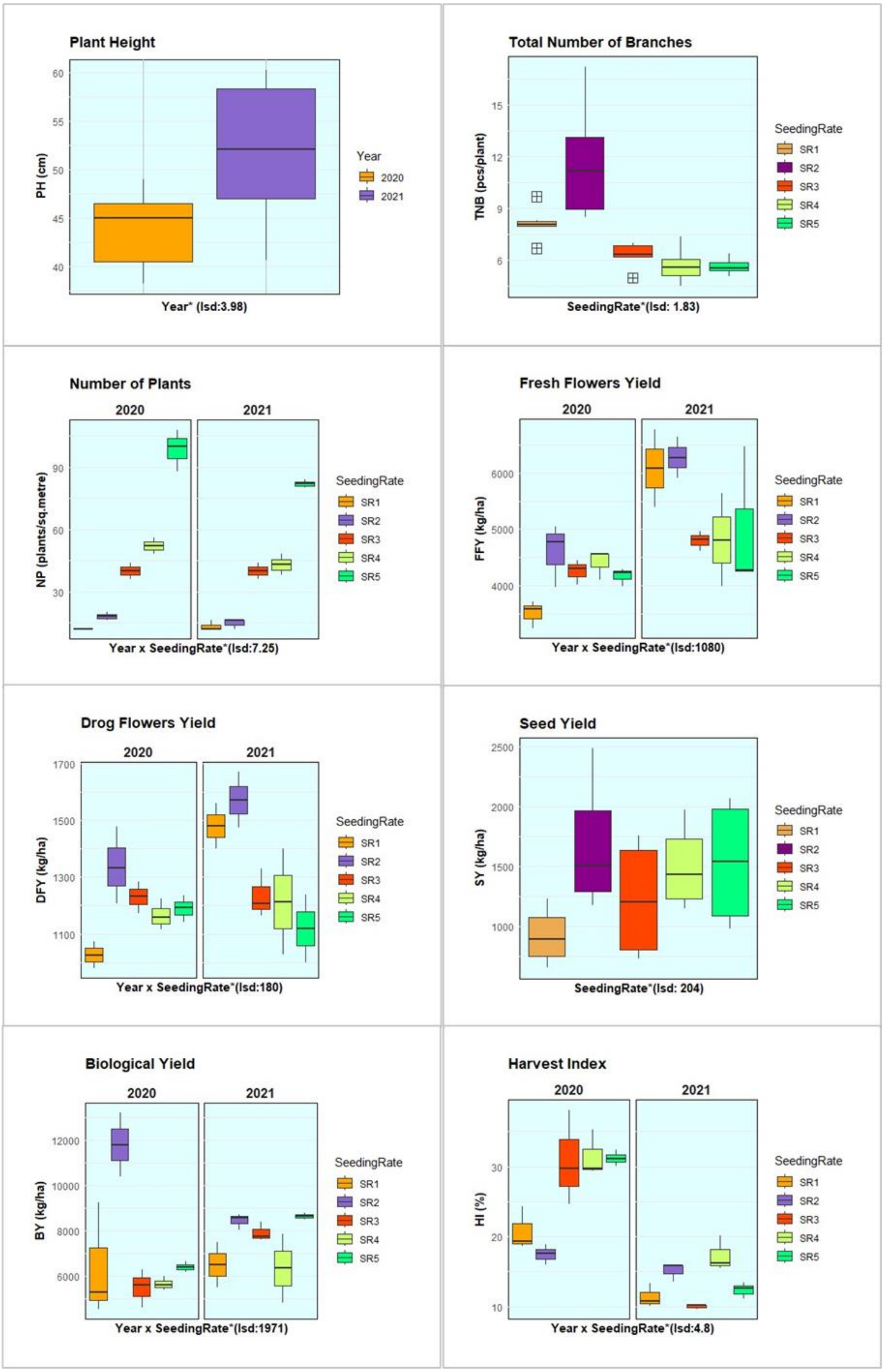

Figure 2. Effects of different seeding rates treatments on morphological and yield parameters *of Calendula officinalis* L.

Quality parameters

Essential oil rate (kg ha^{-1})

The statistical analysis revealed a significant interaction between the year and seeding rates on the essential oil rate ($p<0.05$). Fig. 3 shows that the essential oil content ranged from 0.06% to 0.11%, with the maximum value of 0.11% observed in 2021 at a seeding rate of 25 kg ha^{-1}. In the trial years, no statistically significant difference was observed among the other seeding rates applied. The minimum essential oil rate was identified at 35 kg ha^{-1} seeding rate in the first year. The first year showed more favorable climatic conditions, including temperature and rainfall, for *Calendula officinalis* L., a cool climate plant, compared to the second year. Observations throughout the growing season showed a tendency for the flower to shrink in the second year due to weather conditions (Fig 1). This change in flower size is thought to potentially affect the essential oil rate (Lastra Valdés and Piquet García, 1999).

The essential oil yield of *Calendula officinalis* L. is known to vary depending on cultivation conditions and cultivar characteristics. Research by Raal et al (2016), reported oil yields ranging from 0.10% to 0.43%, with the 'Double Ball' cultivar producing the highest yield. Similarly, studies by Lastra Valdés and Piquet García (1999) and Okoh et al. (2008) demonstrated varied oil yields depending on the plant's part, with fresh flowers yielding 0.09% oil. Additionally, Berimavandi et al. (2011) and Mollafilabi et al. (2014) found that increasing plant density reduced essential oil yield, a trend also observed in our study, where the seeding rate of 35 kg ha^{-1} in the first year resulted in the lowest oil content. Research's findings align with earlier studies, suggesting that essential oil yield is sensitive to environmental factors, plant density, and cultivar differences. The observed decrease in essential oil content at higher plant densities further emphasizes the role of plant arrangement in optimizing oil production, with lower densities potentially leading to better yield outcomes (Shakib et al., 2010). The variation in results across different studies reflects the complexity of essential oil production, which is influenced by a combination of genetic, environmental, and agronomic factors.

Total flavonoid content (mg rutin g^{-1})

Flavonoid levels were analyzed concerning seeding rates over the years. The impact of years on seeding rates was deemed insignificant, whereas the influence of seeding rate treatments proved statistically notable ($p<0.05$). Figure 3 illustrated that the maximum total flavonoid content was achieved at a seeding rate of 35 kg ha^{-1} (72.1 mg rutin g^{-1}), followed closely by 25 kg ha^{-1} (72.0 mg rutin g^{-1}), 15 kg ha^{-1} (71.4 mg rutin g^{-1}), and 10 kg ha^{-1} (66.9 mg rutin g^{-1}) treatments. Specifically, the minimum total flavonoid content was recorded at 5 kg ha^{-1} (ranging from 35.8 to 54.8 mg rutin g^{-1}), with the maximum concentration observed at 10 kg ha^{-1}, totalling 77.8 mg rutin g^{-1} in 2021. On average, the total flavonoid content across treatments amounted to 62.2 mg rutin g^{-1} in 2020 and increased to 68.9 mg rutin g^{-1} in 2021. Notably, a trend emerged where higher seeding rates correlated positively with increased flavonoid content.

Calendula officinalis L. exhibits varying total flavonoid contents across different plant parts. The ligulate ray-florets and tubular disc-florets of *C. officinalis* collectively contain 0.88% and 0.25% of flavonoids, respectively (Masterova, 1991). The peripheral florets of *C. officinalis* contain the maximum flavonoid content at 36.66 mg g^{-1}, followed by tubular florets at 9.95 mg g^{-1}, and leaves at 9.65 mg g^{-1} (Daniil and Kashchenko, 2013). Harvest time significantly influences flavonoid content, with the maximum levels found three days after anthesis, recommending a harvest every three days for optimal yield (Isabela et al., 2016). The total flavonoid content in aqueous-methanolic extracts of *C. officinalis* ranges between 44.91 and 76.44 mg QE g^{-1} dry weight, with rutin, quercetin-3-O-glucoside, and other compounds identified (Rigane et al., 2013). The differences observed in the total flavonoid content across literature studies can be attributed to variations in the applied analysis methods and influencing factors.

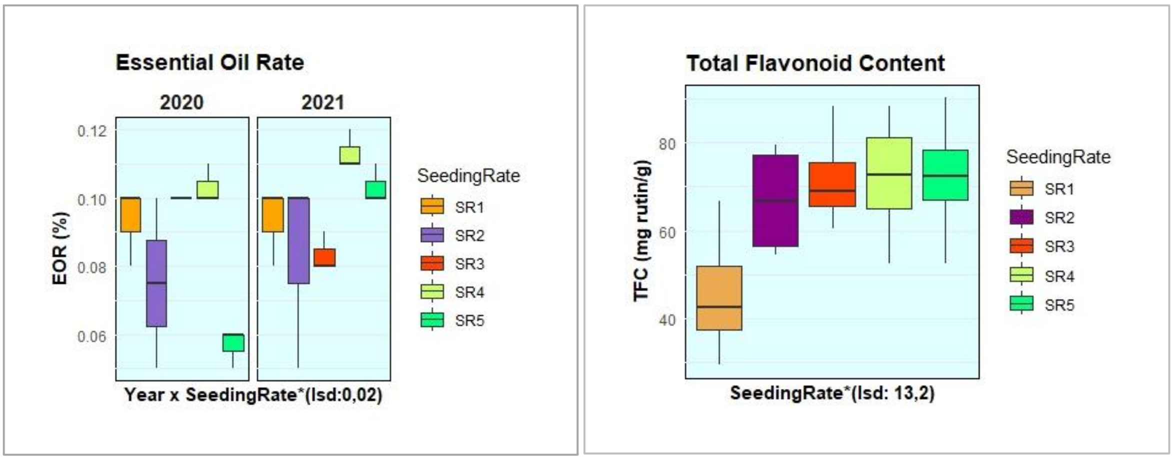

Figure 3. Effects of different seeding rates treatments on quality parameters *of Calendula officinalis* L.

Correlation

A pairwise correlation test was executed to assess the interrelationships among the investigated traits under diverse seeding rate treatments in *Calendula officinalis* L. The correlation table delineating these associations is provided in Figure 4.

The number of plants increased in correlation with the increase at seeding rate (kg ha^{-1}) applied (r^2=0.85, p<0.001). The analysis revealed several significant correlations between the examined traits and seeding treatments in *Calendula officinalis* L. Significant negative correlations were identified between the number of plants and plant height, as well as between the total number of branches and plant height. Conversely, a positive correlation was found between the number of plants and harvest index. The strongest negative relationship was observed between the total number of branches and number of plant (r=-0.55, p<0.05), indicating a decrease in the number of branches with increasing plant density per square meter. Another notable negative correlation was observed between plant height (r=-0.40, p<0.05) and drug flower yield (r=-0.40, p<0.05), suggesting that higher plant density led to decreased plant height and reduced yield of drug flowers. On the other hand, a positive association was noted between the number of plants and harvest index (r=0.37, p<0.05), indicating an increase in harvest index with greater plant density. Additionally, a statistically significant positive relationship was found between the biological yield value and the total number of branches (r=0.67, p<0.05), indicating that as the number of branches increased, so did the biological yield. Furthermore, significant positive correlations were observed between harvest index and seed yield, as well as between harvest index and the number of plants. Conversely, significant negative correlations were found between harvest index and plant height, fresh flower yield, and biological yield. Notably, the strongest negative correlation was observed with plant height (r=-0.69, p<0.05), followed by fresh flower yield (r=-0.53, p<0.05) and biological yield (r=-0.47, p<0.05). The most substantial positive correlation was found between harvest index and seed yield (r=0.73, p<0.05), with another positive correlation noted with the number of plants (r=0.37, p<0.05). The analysis revealed a significant negative correlation (r=-0.46, p<0.05) between the essential oil ratio and the total number of branches, indicating that the essential oil ratio decreased as the total number of branches increased. Additionally, a significant positive correlation (r=0.44, p<0.05) was found between the total flavonoid content and the number of plants, establishing a positive relationship between these traits. This relationship underscores the role of plant density in optimizing secondary metabolite production, suggesting that denser plant populations may induce competitive stress, thereby triggering metabolic pathways associated with flavonoid biosynthesis.

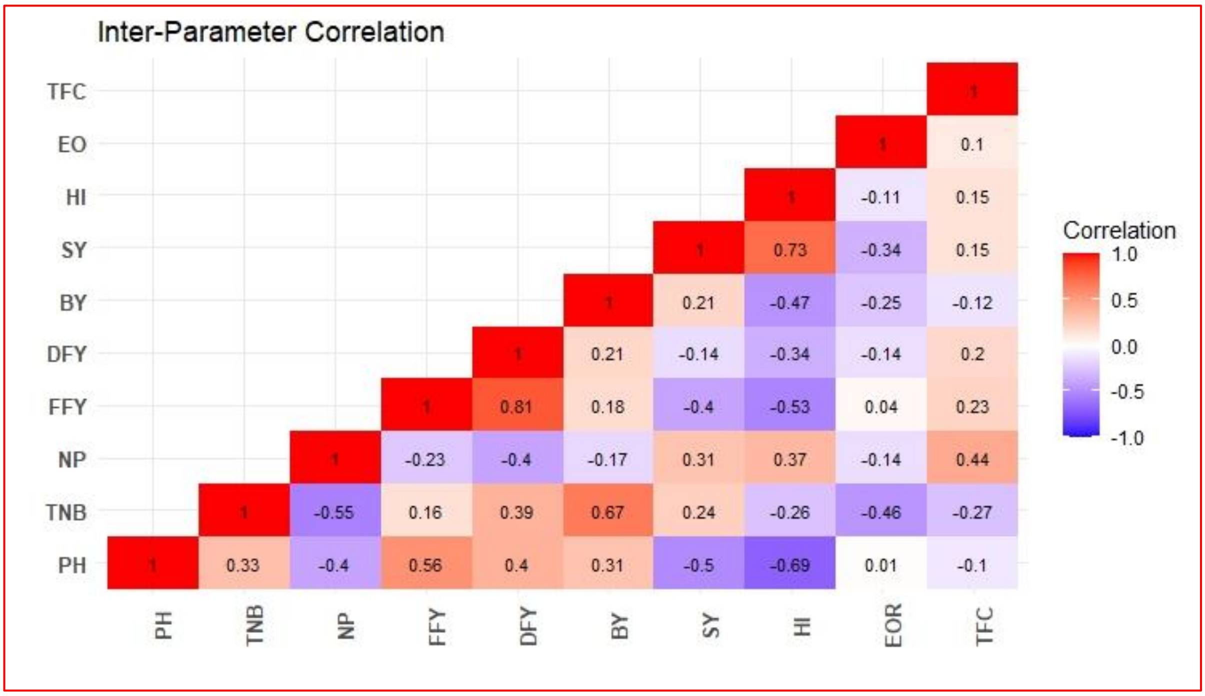

Figure 4. Correlation analysis results for parameters analysed at different seeding rates of *Calendula officinalis* L.

These findings highlight the complex relationship between seeding rate treatments and various traits in *Calendula officinalis* L., revealing how plant growth and yield respond to different conditions. Climate change significantly affects the correlation between seeding rates and seed production. Environmental changes, such as rising temperatures and prolonged droughts, directly impact seed quality and agricultural yield. Regions like Asia and Africa are particularly vulnerable, with projected reductions in agricultural output ranging from 4% to 10% (Sing et al., 2013). Water availability, a critical climate variable, also influences seed development. This necessitates adjustments in seeding strategies to maintain optimal plant density and yield (Bailly et al., 2023). Studies show that varying seeding rates can mitigate some adverse effects of climate change. For instance, higher seeding rates can increase the number of reproductive structures, while lower rates may enhance grain yield per structure (Kuhling et al., 2017). Understanding the interaction between climate change and seeding rates is essential to ensure agricultural productivity and food security in an evolving environmental landscape. However, the success of these strategies depends on local climate conditions and soil management practices (Garnier et al., 2021).

4. CONCLUSION

Significant relationships were identified between seeding rate treatments and the examined traits of *C. officinalis* L. An increase in the seeding rate led to a higher number of plants. This was positively correlated with the harvest index and total flavonoid content. However, traits such as plant height, fresh flower yield, and biological yield decreased with increasing plant density. A similar trend was observed in drug flower yield, which declined as plant density per unit area increased. Considering flower yield, one of the most critical traits, the 10 kg ha^{-1} seeding rate produced the best results. This rate (16.3 plants m^{-2}) allowed for higher branching, likely due to a wider distribution of plants compared to other treatments. The increase in branching contributed to greater plant biomass per unit area. Climate conditions, which varied by year, also had a significant impact. Changes in thermal conditions and vegetative density influenced the optimal seeding rate for *C. officinalis* L., a cool-climate species grown in the Mediterranean region. These findings emphasize the importance of understanding the interplay between climate and agronomic practices to optimize productivity. In conclusion, the 10 kg ha^{-1} seeding rate was determined to be the most suitable for maximizing the agronomic productivity of *Calendula officinalis* L. under Mediterranean climate conditions in Türkiye.

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