Turkish Journal of Field Crops
field-crops.org

DOI: 10.17557/tjfc.1575836
ISSN: 1301-1111
e-ISSN: 3023-6657
2025, 30(1), 192-205

The Study on *Calendula officinalis* L. In Mediterranean Climatic Conditions: Impact of Different Nitrogen and Phosphorus Fertilization Levels on Morphological, Yield and Physiological Characteristics

Gülsüm BOZTAŞ [1]*, Emine BAYRAM [1]

[1] *Ege University, Faculty of Agriculture, Department of Field Crops, 35100, Izmir, Türkiye*

✉ *Corresponding author: gulsumboztas@gmail.com*

ARTICLE INFO

Research Article
Received: 30 October 2024
Accepted: 1 June 2025
Published: 23 June 2025

Keywords:
Calendula officinalis
Fertilization
Nitrogen use efficiency (NUE)
Phosphorus use efficiency (PUE)
Pot marigold
Yield

Citation: Boztas, G., & Bayram, E. (2025). The study on *Calendula officinalis* L. in Mediterranean climatic conditions: Impact of different nitrogen and phosphorus fertilization levels on morphological, yield and physiological characteristics. *Turkish Journal of Field Crops, 30*(1), 192-205.
https://doi.org/10.17557/tjfc.1575836

ABSTRACT

Pot marigold, *Calendula officinalis* L., a plant widely utilized in cosmetics, medicine, and pharmacy due to its rich bioactive compounds, was the subject of this study. The research aimed to investigate the effects of varying nitrogen (0, 50, 100, and 150 kg ha^{-1} N) and phosphorus (0, 50, and 100 kg ha^{-1} P_2O_5) fertilizer doses on the morphological, physiological, and yield-related characteristics of *C. officinalis* cultivated under Mediterranean climatic conditions. Field experiments were conducted during the 2019-2020 and 2020-2021 growing seasons in İzmir Province, western Türkiye, using a randomized complete block design with factorial arrangements and three replications. Morphological traits, seed and flower yields, and nutrient use efficiencies were assessed.

The results revealed that nitrogen and phosphorus doses, year, and their interactions significantly influenced plant growth, yield performance, and nutrient use efficiency. The highest agronomic nitrogen use efficiency based on seed yield (35.7 kg/kg N ha^{-1}) and flower yield (49.3 kg/kg N ha^{-1}) was recorded at 0-50 kg ha^{-1} N in 2020. Similarly, phosphorus use efficiency peaked at 34.5 kg/kg P ha^{-1} under the same conditions but declined sharply in the following year due to reduced and irregular rainfall.

These findings underscore the critical influence of soil moisture on nutrient uptake and utilization. Moderate fertilizer inputs (50 kg ha^{-1} N and 50 kg ha^{-1} P_2O_5) provided a favourable balance between yield and nutrient efficiency. Fertilization strategies for *Calendula officinalis* should be based on integrated evaluations of both yield performance and nutrient use efficiency to ensure sustainable cultivation under Mediterranean agroecological conditions.

1. INTRODUCTION

Pot marigold (*Calendula officinalis* L.) is an annual, or occasionally short-lived perennial, herbaceous plant belonging to the Asteraceae family. Characterized by its aromatic and highly branched stem reaching heights of 15-50 cm, *C. officinalis* has been traditionally used for treating a wide range of diseases, with particular emphasis on its antitumor and cytotoxic activities (Webb et al., 1988; Preethi et al., 2010). The pharmacological significance of this species is recognized by international authorities such as the German Commission E, British Herbal Pharmacopoeia, EMA, ESCOP, and WHO, which document both in vivo and in vitro studies and recent clinical research (Egeli, 2022).

Environmental conditions and agronomic practices substantially influence the production and concentration of secondary metabolites in medicinal plants (Hussain et al., 2011; Berimavandi et al., 2011). Under nutrient stress, plant growth tends to be more constrained by mineral nutrient availability than by photosynthetic capacity (Chapin, 1980). Nitrogen (N) and phosphorus (P) are essential macronutrients that play critical roles in promoting vegetative growth, flowering, and secondary metabolite synthesis (Pandey and Mishra, 2005; Ahirwar et al., 2012). Adequate nitrogen improves vigor and flower quality, whereas phosphorus plays a vital role in various plant metabolic processes, including energy transfer (ATP), nucleic acid synthesis (DNA and RNA), membrane structure (phospholipids), and overall growth regulation (Verma et al., 2017; Defforey and Paytan, 2018; Odoom & Ofosu, 2024). Deficiencies in these nutrients often result in poor plant growth and reduced flowering (Kumar et al., 2015). While appropriate nitrogen fertilization enhances crop yield by increasing leaf area index, photosynthetic duration, and flower formation potential (Hao et al., 2024), excessive nitrogen inputs can alter the biochemical composition of medicinal plants, compromising their therapeutic properties (Rahemi, 2004; Ganjali et al., 2010; Zhu et al., 2023). Thus, determining the optimal nutrient management strategy is critical for maintaining productivity and quality in pot marigold.

Efficient nutrient use is essential for improving crop yields, ensuring fertilizer cost-effectiveness, and reducing environmental impact (Rawal et al., 2022). Nitrogen use efficiency (NUE) refers to the crop yield produced per unit of nitrogen available in the soil (Moll et al., 1982), while phosphorus use efficiency (PUE) represents biomass or yield obtained per unit of phosphorus absorbed. Variations in nitrogen application rates are known to significantly affect both nitrogen uptake and crop yield (Baligar and Barber, 1979). Understanding the agronomic and physiological responses of high-yielding crops to fertilization regimes is critical for advancing breeding efforts and optimizing crop management practices (Yuan et al., 2011; Xue et al., 2013). Improving NUE is particularly important at the production level for balancing economic profitability and environmental sustainability (Cassman et al., 1996; Novoa and Loomis, 1981). Economic optimum nitrogen rates can be determined by considering fertilizer costs, yield responses, and market prices (Hong et al., 2007; Kyveryga et al., 2011; Morris et al., 2018). Recent studies indicate that *C. officinalis* exhibits notable plasticity in nitrogen uptake and biomass accumulation depending on nitrogen levels and harvest timing (Johnson et al., 2018; Marek et al., 2018). While seed yield tends to increase with nitrogen application, NUE declines beyond a certain threshold, suggesting diminishing returns (Serra et al., 2013). Additionally, the combined effects of nitrogen and phosphorus fertilization significantly influence floral characteristics and overall yield (Samoon et al., 2018).

It is imperative to comprehend these aspects to optimize nitrogen and phosphorus management tactics for enhancing the growth and productivity of pot marigold while mitigating environmental risks. This investigation delves into the influence of changing environmental conditions on agronomic nitrogen and phosphorus utilization efficiency to establish suitable fertilization approaches and enhance plant performance for maximizing product output from a unit area of *Calendula officinalis* L.

2. MATERIALS AND METHODS

Experiment site and plant material

This study was conducted over two consecutive years (2019–2020 and 2020–2021) under the coastal Mediterranean climate zone of İzmir, in the experimental fields of the Department of Field Crops, Faculty of Agriculture, Ege University. The experimental material, consisting of seeds of the *Calendula officinalis* L. species, was sourced from a population provided by the PHARMASAAT seed company in Germany. Izmir province, situated in the Mediterranean climate region, lies within the Aegean Region in the western part of Turkey, spanning between 37° 45' and 39° 15' northern latitudes, and 26° 15' and 28° 20' eastern longitudes.

Climatic and soil characteristics

The experimental site displays a soil composition of clay, loam, and alluvial structure. The pH level of the experimental site is 7.88, indicating a slightly alkaline environment. With a water-soluble salt content of 0.091%, there are no hindrances to plant growth. The lime concentration stands at 14.18%, suggesting a moderate presence of lime in the soil. However, the experimental site shows a deficiency in organic matter and total nitrogen, insufficiency in available phosphorus, and an abundance of available potassium. During the years of the study, air temperatures began to increase in March, peaking in August of 2019, July of 2020, and 2021 at 29.8, 29.7, and 30.6 °C, respectively. Conversely, the lowest average temperatures for the three years were observed at 8.7, 8.3, and 10.6 °C in January. The climatic diagram of the experimental field during years in 2019, 2020 and 2021 was given in Figure 1.

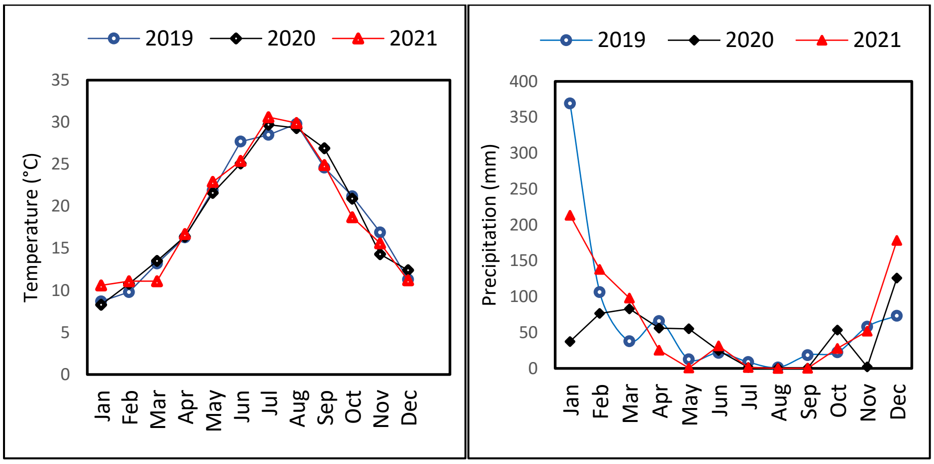

Figure 1. Experimental field climatic diagram

Experimental design and treatments

The study was conducted using a factorial experimental design arranged in randomized complete block design with two main factors. These factors included nitrogen doses (0 [N0], 50 [N5], 100 [N10], and 150 [N15] kg ha^{-1} N) and phosphorus doses (0 [P0], 50 [P5], and 100 [P10] kg ha^{-1} P_2O_5). Fertilizers used in the experiment were Urea with 46% N content and Triple Super Phosphate with 44% P_2O_5. Half of the nitrogen doses were split into two applications: at planting and at the start of flowering. All phosphorus fertilizer was applied to the rows prior to planting. The research spanned two years, from 2019 to 2021.

The study consisted of 36 plots arranged in a factorial design with 4 nitrogen levels, 3 phosphorus levels, and 3 replications. Each plot measured 3.0 meters in length and 1.6 meters in width, with 1.5-meter spacing between blocks and 1.0 meter between nitrogen plots, totaling an experimental area of 266.4 m². Seed sowing was conducted on November 13, 2019, in the first year and on November 12, 2020, in the second year. Seedlings were transplanted into rows spaced 40 cm apart, with 30 cm between plants within rows. Each plot included four rows, totaling 1,440 seedlings across the experiment, with a planting density of 40 plants m^{-2}. Transplanting was carried out on March 19, 2020, and April 8, 2021, respectively. Seedling establishment was supported by irrigation during the first three days after transplanting. Weed control was maintained manually throughout both seasons until seed harvest. Post-planting, irrigation was applied at regular intervals according to plant needs and prevailing weather conditions.

Morphological and yield measurements

Before harvest, plant height (cm), total branch number, and number of flowering branches (per plant) were recorded from ten randomly selected plants in each plot. The seed yields were determined using the first and fourth rows of the trial, while the second and third rows were allocated for drug flower yields (kg ha^{-1}). Following the initiation of flowering in the trial period (April 12, 2020, and May 4, 2021), the flowers in the remaining section were collected and weighed weekly from the onset to the conclusion of flowering after removing edge effects from the 2nd and 3rd rows of each plot. Subsequently, the fresh flower yield (kg ha^{-1}) was computed. The plants with quantified fresh flower yields underwent a drying process in a drying cabinet at 30 °C for 3-4 days. The seeds from the plant samples were then isolated and weighed, and the seed yield (kg ha^{-1}) was ascertained.

Physiological measurements

Nitrogen Use Efficiency (NUE) was determined by analysing seed and flower yield data collected from each plot using the agronomic efficiency measure [(yield in kilograms per kilogram of nitrogen input)] using the following formula:

 NUE_{seed} (kg/kg N ha^{-1}): (TN-T0)/ΔN

TN: Seed yield obtained from nitrogen application, kg

T0: Seed yield obtained from control (0 kg N ha^{-1}) application, kg

ΔN: Difference between nitrogen applications, kg

 NUE_{flower} (kg/kg N ha^{-1}): (TN-T0)/ΔN

TN: Drug flower yield obtained from nitrogen application, kg

T0: Drug flower yield obtained from control (0 kg N ha^{-1}) application, kg

ΔN: Difference between nitrogen applications, kg

Phosphour Use Efficiency (PUE) was determined by analyzing seed and flower yield data collected from each plot using the agronomic efficiency measure [(yield in kilograms per kilogram of phosphorus input)] using the following formula:

 PUE_{seed} (kg/kg P ha^{-1}): (TP-T0)/ΔP

TP: Seed yield obtained from phosphorus application, kg

T0: Seed yield obtained from control (0 kg P_2O_5 ha^{-1}) application, kg

ΔP: Difference between phosphorus applications, kg

 PUE_{flower} (kg/kg P ha^{-1}): (TP-T0) /ΔP

TP: Drug flower yield obtained from phosphorus application, kg

T0: Drug flower yield obtained from control (0 kg P_2O_5 ha^{-1}) application, kg

ΔP: Difference between phosphorus applications, kg

Statistical analysis

The statistical analysis was performed using the software JMP 14® by SAS Institute, Inc. (2018). Significantly different fertiliser treatments were examined through a two-way analysis of variance (ANOVA) with mean separation conducted using a least significant difference (LSD) test ($p<0.05$). Boxplots grouping was carried out in R Studio utilizing the 'tidyverse' (Wickham et al., 2019), 'ggplot2' (Wickham et al., 2016), and 'gapminder' (Bryan, 2022) packages.

3. RESULTS AND DISCUSSION

Morphological characteristics

Plant height

Plant height was significantly influenced by the interaction between year and nitrogen fertilization ($p<0.01$) (Fig. 2). The maximum plant height was recorded in the first year at a nitrogen level of 50 kg ha^{-1} (58.2 cm), whereas in the second year, the tallest plants (38.6 cm) were observed at 150 kg ha^{-1} N. Nitrogen application enhanced plant height in both years; however, the optimal nitrogen rate varied between seasons, indicating a strong dependency on annual environmental conditions such as temperature, soil moisture, and precipitation patterns. Seasonal variability also affected phosphorus responsiveness. While the highest plant height in 2020 was achieved at 50 kg ha^{-1} P_2O_5 (56.8 cm), phosphorus fertilization had no statistically significant effect on plant height in 2021 ($p>0.05$), likely due to limited root activity and impaired nutrient translocation under drought conditions.

Drought restricts nitrogen use efficiency through several physiological mechanisms. One of the most critical is stomatal closure, which limits CO_2 uptake and reduces photosynthetic capacity, thereby decreasing the nitrogen requirement for metabolic activities (Ullah et al., 2019; Zhang, 2024). Given the central role of nitrogen in chlorophyll synthesis and photosynthesis, disruptions to these processes under water-deficit conditions constrain biomass accumulation and overall plant growth (Ye et al., 2022). In addition, drought stress negatively impacts root development and function, both of which are essential for efficient nitrogen uptake. A reduction in root surface area and volume impairs nitrogen acquisition from the soil (Wang et al., 2024). Drought conditions also modify the composition of root exudates, which play a crucial role in nutrient mobilization within the rhizosphere, thereby further inhibiting nitrogen uptake (Wang et al., 2024).

Although supplemental irrigation was implemented during both growing seasons, extremely low rainfall during critical developmental stages-particularly in May 2021 (0.6 mm compared to 55.2 mm in May 2020)-likely intensified water stress, reducing nitrogen mobility in the soil and limiting its biological availability (Fig. 1). This highlights the limitation of irrigation in fully compensating for severe drought stress, especially in Mediterranean climates where rainfall distribution and timing are key factors for supporting vegetative growth. In 2020, favorable precipitation enabled efficient nitrogen utilization at moderate application rates, resulting in increased plant height. In contrast, in 2021, despite higher nitrogen inputs, reduced growth was observed, suggesting not an increased nitrogen requirement but a diminished capacity for nitrogen absorption and assimilation under drought conditions.

Overall, the benefits of nitrogen fertilization on plant height appear to be significantly constrained under drought, primarily due to physiological limitations in uptake and utilization rather than nitrogen deficiency per se. These findings are consistent with earlier reports. Moosavi et al. (2014) demonstrated that drought stress reduces biomass and plant height by impairing photosynthetic activity and altering assimilate distribution. Rahmani et al. (2008) also reported that drought during early vegetative stages significantly suppresses plant height in *Calendula officinalis*. Similarly, Kwiatkowski et al. (2020) noted that while nitrogen levels between 60 and 80 kg ha^{-1} enhanced plant height, morphological responses were strongly influenced by seasonal weather conditions and water availability.

Total number of branches

The interaction between year, nitrogen dose, and phosphorus dose had a statistically significant effect on the total number of branches in *Calendula officinalis* L. ($p<0.01$) (Fig. 2). The highest number of branches was recorded in the first year under the 50 kg ha^{-1} N × 100 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} N × 100 kg ha^{-1} P_2O_5 fertilizer combinations. In contrast, the lowest branching (5.07 branches per plant) was observed in the second year at 0 kg ha^{-1} N × 50 kg ha^{-1} P_2O_5. These results suggest that both nitrogen and phosphorus application promote vegetative branching in *C. officinalis*, although the magnitude of response varied between growing seasons.

Comparative findings in the literature support these observations. Kumar and Singh (2011), in a study conducted under Indian conditions, reported the highest total branch number (20.7 branches per plant) at a nitrogen dose of 150 kg ha^{-1}. Other studies have documented a broad range in branching capacity: Kareem et al. (2014) reported values between 3.67 and 15.3, while Pazoki et al. (2016) found a range of 5.7 to 13.0 branches per plant. Additionally, Doğramacı and Arabacı (2010) observed that both organic and inorganic fertilizers positively influenced branching in *Pimpinella anisum*.

Although the branch numbers recorded in the present study were lower than those reported by Kumar and Singh (2011), they align closely with the ranges observed by Kareem et al. (2014) and Pazoki et al. (2016). These variations in branching responses may be attributed to differences in environmental conditions, soil fertility, genotype, and fertilizer timing, emphasizing the importance of site-specific management in optimizing vegetative growth traits in *Calendula officinalis*.

Number of flowering branches

A statistically significant three-way interaction was observed among year, nitrogen dose, and phosphorus dose on the number of flowering branches in *Calendula officinalis* L. ($p < 0.01$) (Fig. 2). The number of flowering branches varied across phosphorus levels depending on nitrogen doses and growing seasons. The highest number of flowering branches per plant (89.0) was recorded in 2021 under the 150 kg ha^{-1} N × 50 kg ha^{-1} P_2O_5 treatment, while the maximum value in 2020 (83.2) was observed with the 50 kg ha^{-1} N × 50 kg ha^{-1} P_2O_5 combination. The lowest number of flowering branches (52.9) was noted in the control (unfertilized) treatment in 2020. When yearly averages were considered, the second year yielded a higher average number of flowering branches (79.5 per plant) compared to the first year (65.0 per plant). This suggests that the response to nitrogen fertilization was more pronounced in 2021, potentially due to more favourable environmental conditions. However, although the number of flowering branches increased in the second year, a reduction in flower diameter was also observed, indicating a potential trade-off between branch quantity and flower size (Fig. 2).

Previous studies have reported a wide range of flowering branch numbers in *C. officinalis* depending on fertilizer regimes and environmental factors. For instance, Nagmote et al. (2020) reported a maximum of 55.16 flowering branches per plant under 100 kg ha^{-1} N × 50 kg ha^{-1} P application, while Kumar et al. (2015) observed fewer branches (35.7) with 150 kg ha^{-1} N. Other researchers reported even lower figures: Crnobarac et al. (2009) found approximately 30 branches, Ganjali et al. (2010) noted 38.29-45.83, and Król (2011) reported 41.6-69 flowering branches per plant.

The discrepancies between the literature findings and our results regarding this particular characteristic can be attributed to factors such as environmental conditions, variations in growing seasons, the genetic diversity of the plant materials (population or variety), and discrepancies in the experimental variables examined.

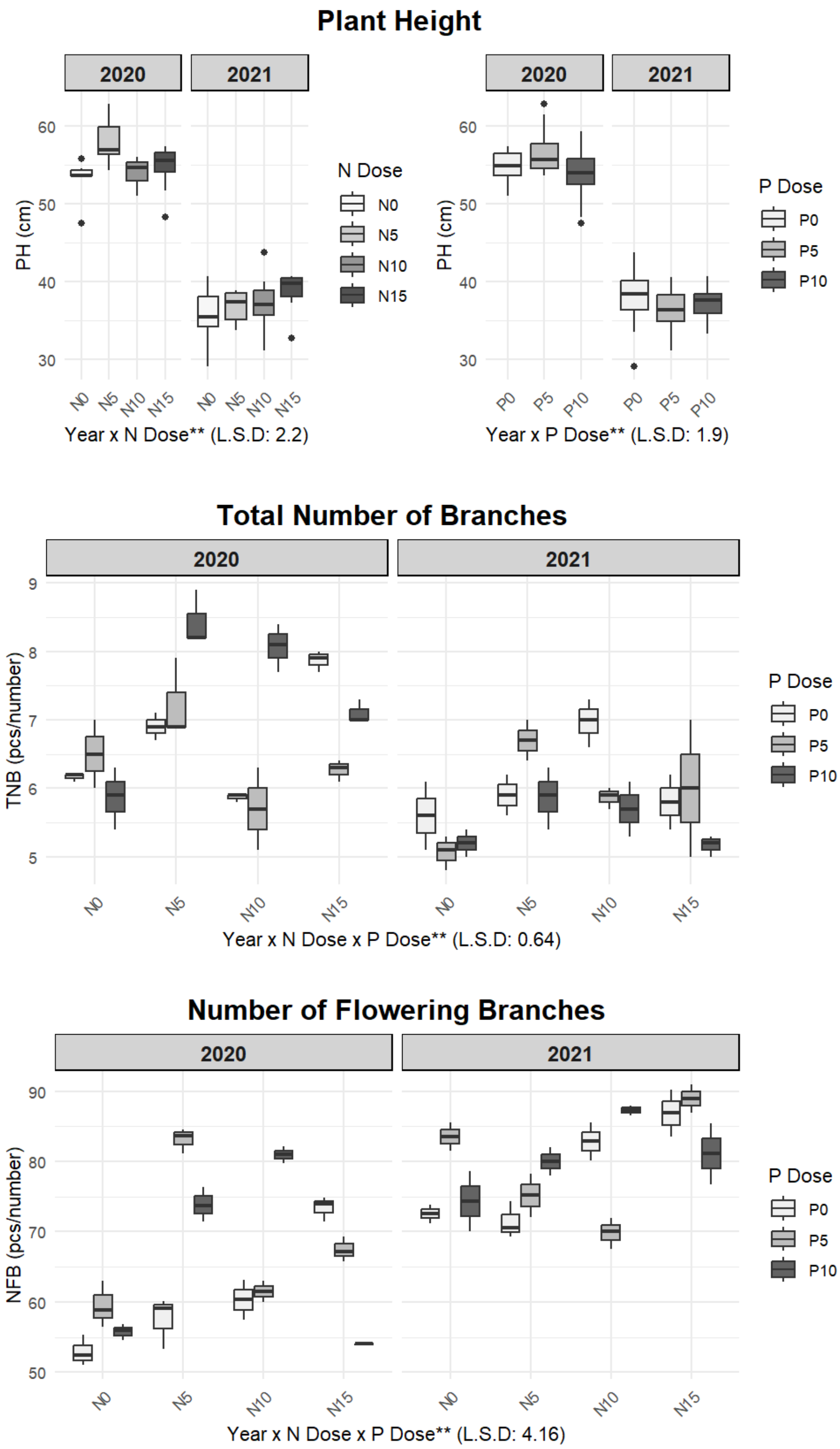

Figure 2. The effect of nitrogen and phosphorus fertilization on morphological characteristics of *Calendula officinalis* L. during 2020 and 2021 years. (* significant at p<0.05 level, ** significant p<0.01 level)

Yield characteristics

Fresh flowers yield

Figure 3 illustrates that the three-way interaction between year, nitrogen, and phosphorus levels significantly influenced fresh flower yield ($p<0.01$). The highest yield was recorded in 2020, with 4.812 kg ha^{-1} under the combined application of 50 kg ha^{-1} N and 50 kg ha^{-1} P_2O_5, while the lowest yield (2.177 kg ha^{-1}) was observed in the unfertilized control treatment. The overall average fresh flower yield across treatments was 3.163 kg ha^{-1}. In the first year, fresh flower yields ranged from 2.551 to 4.812 kg ha^{-1}, whereas in the second year they ranged between 2.177 and 3.741 kg ha^{-1}, indicating a general decline in yield during the second year. This decline was attributed to less favorable climatic conditions. Notably, the fertilizer combination of 50 kg ha^{-1} N × 50 kg ha^{-1} P_2O_5 consistently produced the highest fresh flower yields across both growing seasons, while control plots resulted in the lowest values.

These findings are supported by previous studies that have documented yield increases with increasing nitrogen inputs. Mollafilabi et al. (2014) reported the highest fresh flower yield at a nitrogen dose of 90 kg ha^{-1}. Similarly, Moosavi et al. (2014) observed a 36.8% increase in yield as nitrogen application increased from 0 to 180 kg ha^{-1} (ranging from 4.581 to 6.269 kg ha^{-1}). Under Türkiye agroecological conditions, Akkoç (2021) reported fresh flower yields between 2.486 and 5.019 kg ha^{-1}, with the highest yield achieved at 150 kg ha^{-1} N.

Changes in environmental factors, such as temperature, precipitation, and soil moisture, have the potential to impact both the growth and yield of plants. Variations in yield might be attributed to fluctuations in temperature and rainfall patterns observed between consecutive years. Moreover, the composition of the soil and its fertility levels could also play a role in the variability of yields. Several research studies cited underscore the significance of the amount of fertilizer applied in relation to the yield of fresh flowers. The findings suggest that an optimal amount of fertilizer, for instance 90 kg ha^{-1} N or 150 kg ha^{-1}, can exert a considerable influence on the yield outcomes. Furthermore, the timing of fertilizer application during different growth stages is another factor that could affect the yield results.

Drug flowers yield

According to the results obtained from the drug flower yield, the effect of the trial years on the fertilizer combinations was found to be significant ($p<0.05$) (Fig. 3). When the values of the year × nitrogen × phosphorus interaction are examined, the highest value in drug flower yield was found in 50 kg ha^{-1} N × 50 kg ha^{-1} P_2O_5 application in 2020 (1.320 kg ha^{-1}), whereas the lowest yield was obtained in control application in 2021 (587 kg ha^{-1}). In the second year, the average drug flower yield decreased by approximately 30% compared to the first year. Depending on climatic factors, this situation is thought to be caused by rainfall during the flowering period, which is critical for plant development. Although the average drug flower yield varied in the trial years, the highest values were obtained from 50 kg ha^{-1} N and 50 kg ha^{-1} P_2O_5 applications in both years (Fig. 3).

In fertilization studies conducted on pot marigold, several researchers have reported that drug flower yield increases with rising nitrogen doses (Al-Badawy et al., 1995; Krol, 2011; Kumar et al., 2015; Samoon et al., 2018). However, other studies have indicated that this response plateaus beyond a certain nitrogen threshold, with no further yield enhancement observed (Nagmote et al., 2020; Akkoç, 2021). When the results of the present study are compared with those reported under different cultivation techniques, the flower yields obtained here are notably higher (Ayran, 2017; Yetis, 2019). This discrepancy may be attributed to various factors, including regional climatic conditions, soil properties, differences in cultivation practices, and genetic variation among plant materials.

Seed yield

As shown in Fig. 3, the two-way interactions between year × nitrogen dose and year × phosphorus dose significantly affected seed yield ($p<0.01$). The average seed yield across the year × nitrogen interaction was 688 kg ha^{-1}. Within this interaction, the highest seed yield was obtained from the 100 kg ha^{-1} N treatment in the first year (884 kg ha^{-1}), closely followed by the 50 kg ha^{-1} N treatment (876 kg ha^{-1}). Conversely, the lowest yield was observed in the second year under the control (0 kg ha^{-1} N) condition, with 476 kg ha^{-1}.

Regarding the year × phosphorus interaction, seed yields reached 902 kg ha^{-1} and 893 kg ha^{-1} in the 100 and 50 kg ha^{-1} P_2O_5 treatments, respectively, in 2020. The lowest seed yield (524 kg ha^{-1}) was recorded in the control treatment of the second year. On average, seed yield was 824 kg ha^{-1} in the first year and declined to 552 kg ha^{-1} in the second year, corresponding to a 33% reduction. These results emphasize the impact of both fertilizer input and seasonal conditions on seed productivity in *Calendula officinalis* (Fig. 3).

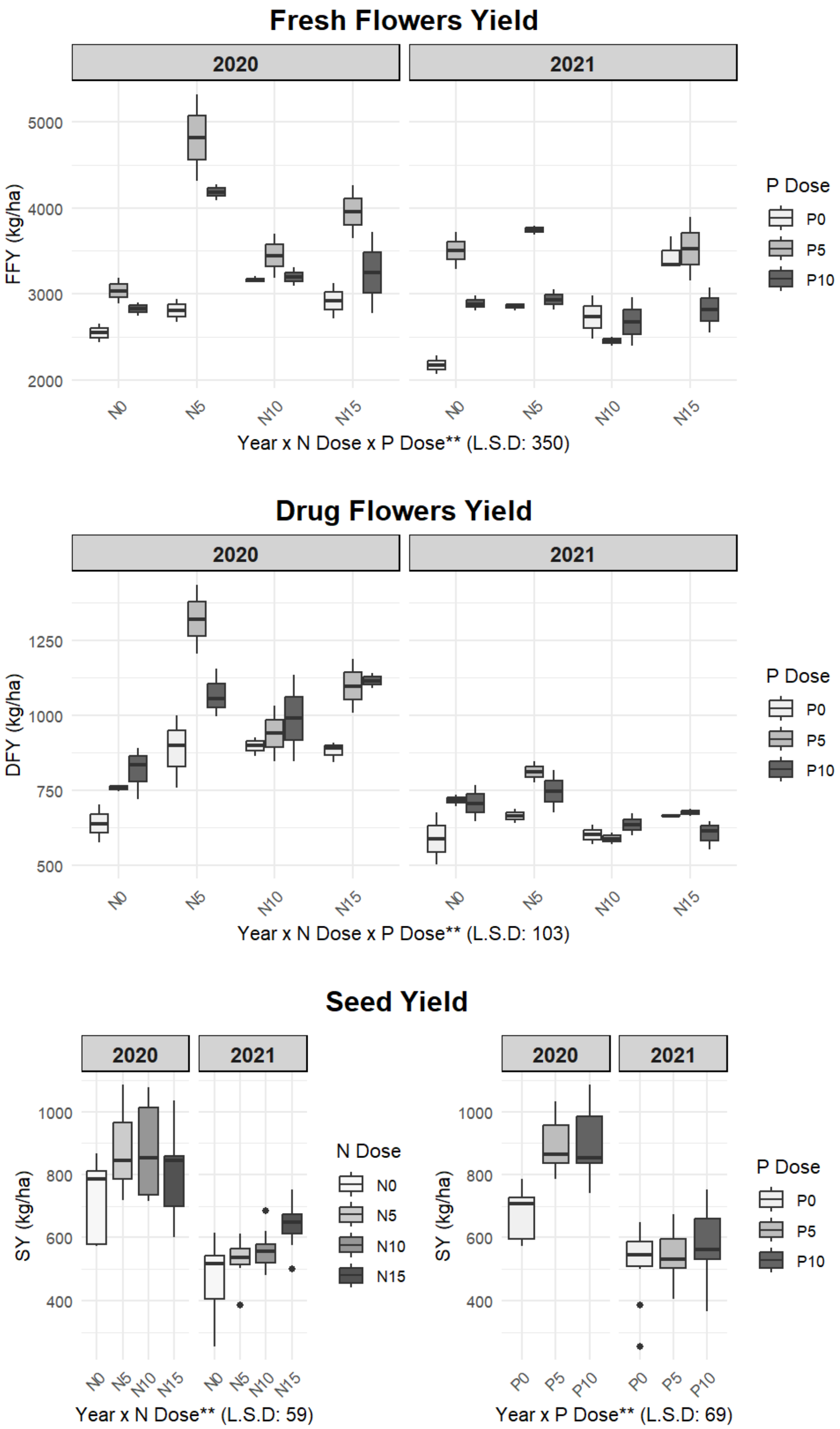

Figure 3. The effect of nitrogen and phosphorus fertilization on yield characteristics of *Calendula officinalis* L. during 2020 and 2021 years. (** significant p<0.01 level)

Seed yield is influenced by numerous environmental and agronomic factors, particularly nitrogen and phosphorus fertilization. Król (2017) reported that the highest seed yields were obtained in seasons with moderate and well-distributed rainfall, particularly with nitrogen applications ranging from 60–90 kg ha^{-1}. Similarly, Rahmani et al. (2011) observed that the application of 90 kg ha^{-1} N led to the highest yield under drought conditions. Moreover, combined N × P applications were found to be effective in maximizing seed yield, with Samoon et al. (2018) and Kumar et al. (2015) recording the highest seed yields at 150 kg ha^{-1} N × 80 kg ha^{-1} P_2O_5. In another study, Jevdovic et al. (2013) reported a yield of 672.84 kg ha^{-1} with 400 kg ha^{-1} NPK, while significantly lower values were obtained under unfertilized conditions.

In the present study, both fertilizer dose and growing season significantly influenced seed yield, with nitrogen application playing a particularly prominent role. Our results support earlier findings indicating that nitrogen fertilization mitigates drought stress by enhancing root development, chlorophyll synthesis, and overall plant physiological performance. While water stress adversely affected yield components, adequately irrigated conditions promoted significantly higher seed yields, reinforcing the importance of water availability alongside optimal fertilization for maximizing *C. officinalis* productivity under Mediterranean conditions.

Physiological characteristics

Nitrogen Use Effenciency$_{Seed}$ (NUE$_{seed}$)

The effects of nitrogen doses and year interaction on nitrogen use efficiency (NUE_{seed}) were found to be statistically significant. The highest NUE_{seed} was determined to be 35.7 kg/kg N ha^{-1} between 0 and 50 kg ha^{-1} nitrogen doses in the first year. In the first year, it was seen that nitrogen use efficiency decreased in response to increasing nitrogen doses at 0-50 kg N ha^{-1} compared to 50-100 and 100-150 kg N ha^{-1} doses. NUE_{seed} values for 2021 decreased significantly compared to 2020 (Fig. 4).

When the NUE_{seed} values of the trial years were examined, it was seen that 2021 decreased significantly compared to 2020 (Fig. 4). As a result of this situation, increasing nitrogen doses in the pot marigold in 2020 reduced the efficiency of nitrogen use and did not make a significant difference in 2021. Nitrogen, due to its structure, dissolves better in soils that are saturated with water and is used more effectively by the plant. Dry conditions not only reduce plant growth and yield but also reduce water infiltration below the rooting zone, nutrient leaching, and losses by surface runoff, possibly increasing nutrient use efficiency (Ullah et al., 2019). In contrast, nutrient uptake and yield in wetter conditions are higher, but the potential for nutrient leaching also increases, reducing nutrient use efficiency (Rupp et al., 2021). When the rainfall amount in April, May, and June of the trial years was examined, it was seen that 2020 was higher than 2021. In this context, it was concluded that the amount of precipitation depending on the year factor was effective in the change in NUE values (Fig. 1).

Nitrogen Use Effenciency$_{flower}$ (NUE$_{flower}$)

The effects of nitrogen dose and year factors on NUE_{flower} were found to be significant. During the research years, the highest value was obtained in 2020 with 30.5 kg/kg N ha^{-1}. It was determined that the average nitrogen use efficiency in 2021 decreased by approximately 76% compared to 2020. The average NUE_{flower} value was 18.9 kg/kg N ha^{-1}, and the highest value was detected at 0-50 kg ha^{-1} nitrogen fertilizer dose (32.3 kg/kg N ha^{-1}). As with NUE_{seed} value, increasing nitrogen doses resulted in a decrease in nitrogen use efficiency. Although the interaction of year x nitrogen dose was not found to be statistically significant, it was observed that the highest NUE_{flower} value among nitrogen doses in the first trial year was obtained at a nitrogen dose of 0-50 kg ha^{-1} (49.3 kg/kg N ha^{-1}) (Fig. 4).

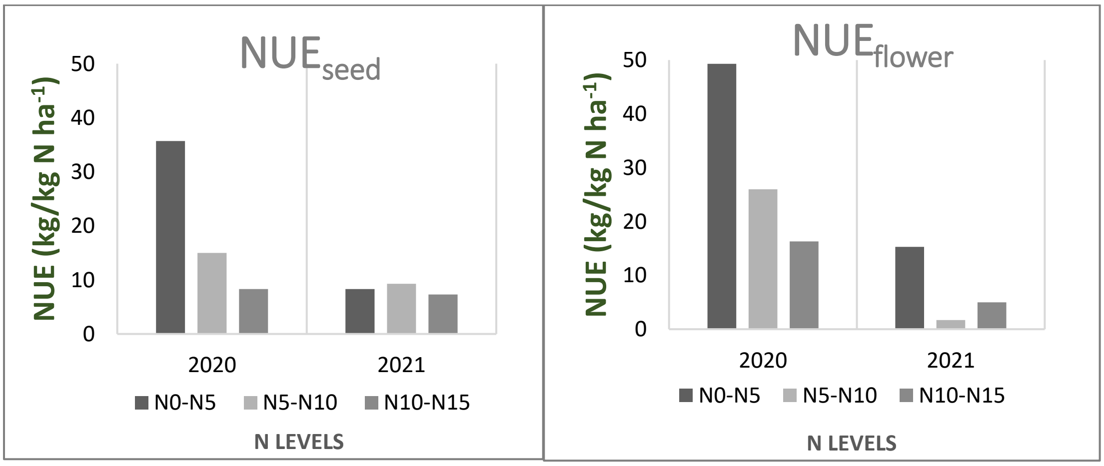

Figure 4. The effects of different nitrogen fertilizers on NUE_{seed} and NUE_{flower} in *Calendula officinalis* L. during 2020 and 2021.

Available water content, which is one of the factors limiting nitrogen use efficiency, is important for soil saturation (Gupta et al., 2012). Rainfall, which is especially effective in the spring period when nitrogenous fertilizers are applied and cover the first development stages of plants, increases the field water capacity and ensures that nitrogen reaches the plant effectively (Ahmad et al., 2014). Considering the trial years of NUE_{flower}, it was seen that the values for 2020 were higher than 2021. When evaluated in terms of the effect of the year factor on NUE_{flower}, it is thought that the resulting difference depends on climatic conditions. When the rainfall values of April, May, and June, which are critical for the growth and development of pot marigold in both trial years, were compared, the total rainfalls were 136.2 and 57.4 mm in 2020 and 2021, respectively. Precipitation values, which showed a more regular course in the first trial year, showed an irregular distribution in the second year (Fig. 1). It can be said that this precipitation change seen in the trial years in the spring had an impact on NUE_{flower} values and limited nitrogen use efficiency in the second trial year, which was relatively dry (Statham, 2012; Nematpour et al., 2019; Raza et al., 2019a). It has been observed that drought stress during the reproductive phase causes a shortening of the time between seed formation and pollen shedding and therefore a decrease in grain filling time (Shil & Dewanjee, 2022).

Phosphour Use Effenciency$_{Seed}$ (PUE_{seed})

In terms of PUE calculated based on seed yield, the 2020 value (34.5 kg/kg P ha^{-1}) was the year in which the highest results were achieved. The average PUE_{seed} value for 2021 was recorded at 3.0 kg/kg P ha^{-1} (Fig. 5). Limiting water needs in plants during their development periods is important for the effective use of phosphorus. In the second trial year of the study, it is thought that the insufficient amount of precipitation in the months covering this period caused a decrease in phosphorus use efficiency (Meisinger and Delgado, 2002).

The effect of the year factor on PUE_{seed} was found to be statistically significant, and the values obtained in the two trial years differed greatly. It is thought that the amount of precipitation due to climatic factors during the trial years was effective in this situation. Plants absorb most of the phosphorus they need at the beginning of their development period, and their uptake can be limited due to factors such as soil pH, structure, moisture content, and climatic factors effective throughout the season (Ullah et al., 2019). When the total precipitation values in January and June of the research years are examined (Fig. 1), the amount of precipitation, which had a regular distribution until June in 2020, was largely effective in January and February in 2021 and then tended to decrease. Although the amount of precipitation in 2021 is higher than the precipitation in 2020 and the long-term average, it has decreased significantly, especially compared to the precipitation that is effective in the spring period. Considering that phosphorus fertilizers were applied with planting in March (2020) and April (2021) in the trial, it is thought that the water content in the soil was effective on phosphorus uptake and the soil water content caused changes in PUE_{seed} values (Kacar, 2020; Scaini et al., 2023).

Phosphorus is found to be an immobile element in the soil. In order for plants to take it up, phosphorus in the soil must be transported to the root area. However, this transport process depends on the presence of water. Water is located in the spaces between soil particles, and these spaces are used by the roots of plants. As the amount of water decreases, the space volume between soil particles decreases. In this case, it becomes difficult for water to reach the roots, and therefore, it becomes difficult for the roots to absorb phosphorus. This means that water is held so strongly in the soil that it cannot be taken up by the roots of plants. In this case, the roots of the plants can hardly take in water, and phosphorus uptake becomes difficult. Decreasing the amount of water negatively affects phosphorus transport in the soil and reduces the phosphorus use efficiency of plants (Kacar, 2020).

Phosphour Use Effenciency$_{Flower}$ (PUE_{flower})

Based on flower production, phosphorus use efficiency was investigated, and it was found that there was no significant relationship between PUE and the year, phosphorus dose, and year × phosphorus interaction. However, compared to 50-100 kg ha^{-1} P_2O_5 fertilizer dose administration in both years, PUE_{flower} values between 0-50 kg ha^{-1} P_2O_5 doses increased the consumption efficiency (Fig. 5).

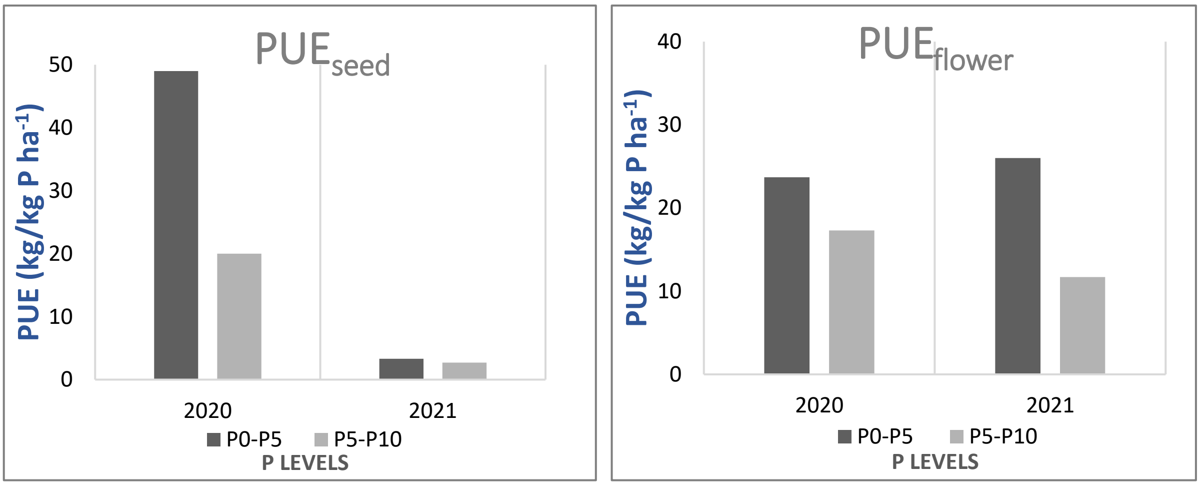

Figure 5. The effects of different phosphorus fertilizers on PUE$_{seed}$ and PUE$_{flower}$ in *Calendula officinalis* L. during 2020 and 2021.

The investigation conducted by Wang et al. (2011) delves into the effects of different levels of phosphorus application on crop yield, water consumption, and nitrogen use efficiency in dryland regions of the loess plateau across varying precipitation patterns. In these areas, the inadequacy of soil phosphorus poses a barrier to crop productivity and the effective utilization of water and nitrogen resources. It is imperative to apply phosphorus fertilizers appropriately to boost crop yield and enhance water and nitrogen use efficiency in dryland farming. The findings indicate an overall increase in most agricultural parameters at lower phosphorus rates, with a subsequent decrease at higher rates, except for water consumption and harvesting index (HI). Elevated phosphorus levels have been shown to have adverse effects on crop yield, water usage, and nitrogen use efficiency in both precipitation scenarios, underscoring the recommendation for a moderate application to achieve optimal productivity and resource utilization. Furthermore, even at moderate levels, phosphorus application leads to a reduction in HI and water consumption compared to non-phosphorus treatments during dry years, emphasizing the significance of implementing balanced fertilization practices.

In order to effectively use phosphorus fertilizers, Kacar (2020) lists a number of elements that must be taken into consideration. These include careful assessment of the qualities of the soil, balanced nutrient use, optimal plant selection, water management, and increased microbial activities. Assessing the aforementioned variables regarding calendula plant phosphorus uptake will aid in obtaining precise data regarding the degree and conditions of PUE$_{flower}$ alterations.

4. CONCLUSION

This study demonstrated that *Calendula officinalis* L., a medicinal and aromatic plant of high therapeutic and economic value, responds significantly to nitrogen and phosphorus fertilization under Mediterranean conditions. Agronomic parameters such as flowering branch number, drug flower yield, seed yield, and nutrient use efficiency (NUE and PUE) were strongly influenced by fertilizer dose and seasonal variation, particularly rainfall. Key findings highlighted that the highest agronomic nitrogen use efficiency (NUE) based on seed and flower yield was achieved at 0-50 kg ha^{-1} N in 2020, with values of 35.7 kg/kg N ha^{-1}and 49.3 kg/kg N ha^{-1}, respectively. Similarly, PUE peaked at 34.5 kg/kg P ha^{-1}in the same year and dose level. However, NUE and PUE decreased considerably in 2021 due to lower and irregular rainfall, indicating the strong influence of environmental conditions -especially rainfall during flowering-on nutrient uptake efficiency. Although the highest nutrient use efficiencies were recorded at the lowest nitrogen and phosphorus levels, this aligns with the well-established agronomic principle that NUE and PUE tend to decrease as nutrient input increases. In conclusion, *C. officinalis* does not require high nutrient inputs to reach its agronomic potential. The application of 50 kg ha^{-1} nitrogen and 50 kg ha^{-1} phosphorus can be recommended as an efficient and environmentally sound fertilization strategy for sustainable production of *C. officinalis* in the Aegean region and other areas with similar agroecological conditions.

ACKNOWLEDGEMENT

This article is a part of Gülsüm BOZTAŞ's PhD thesis carried out at Ege University in Izmir titled "Effects of Different Agro-technical Practices on Yield, Quality and Physiological Characteristics of *Calendula officinalis* L".

REFERENCES

Ahirwar, M. K., Ahirwar, K., & Shukla, M. (2012). Effect of plant densities, nitrogen and phosphorus levels on growth, yield, and quality of African marigold. *Annals of Plant Soil Research*, *14*(2), 153–155.

Akkoc, G. (2021). Effect of nitrogen doses on agronomic characteristics of marigold (*Calendula officinalis* L.) Master's Thesis. Siirt University, Graduate School of Natural and Applied Sciences. 48p.

Al-Badawy, A. A., Abdalla, N. M., & El-Sayed, A. A. (1995). Response of *Calendula officinalis* L. plants to different nitrogenous fertilizers. *HortScience*, *30*(4), 858.

Aslam, A., Zaman, F., Qasim, M., Ziaf, K. H., Shaheen, I., Afzal, N., Ul-Ain, Q., Hussain, S., & Hussain, S. H. (2016). Impact of nitrogen and potash on growth, flower and seed yield of African marigold (*Tagetes erecta* L.). *Scientia Agriculturae*, *14*(2), 266–269. DOI: 10.15192/PSCP.SA.2016.14.2.266269

Ayran, I. (2017). The determination of effect on agronomic characteristics of different sowing of marigold (Calendula officinalis L.) which cultivated in Konya ecological condition Master's Thesis. Selcuk University Institute of Science and Technology. 57p.

Baligar, V. C., & Barber, S. A. (1979). Genotypic differences of corn for ion uptake. *Agronomy Journal*, *71*, 870–873.

Berimavandi, A. R., Hashemabadi, D., Ghaziani, M. V. F., & Kaviani, B. (2011). Effects of plant density and sowing date on the growth, flowering, and quantity of essential oil of *Calendula officinalis* L. *Journal of Medicinal Plants Research*, *5*(20), 5110–5115.

Bryan, J. (2022). Gapminder: Data from Gapminder. R package version 0.3.0. https://doi.org/10.5281/zenodo.594018.

Cassman, K. G., Gines, G. C., Dizon, M. A., Samson, M. I., & Alcantara, J. M. (1996). Nitrogen-use efficiency in tropical lowland rice systems: Contributions from indigenous and applied nitrogen. *Field Crops Research*, *47*(1), 1–12.

Chapin III, F. S. (1980). The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, *11*(1), 233–260.

Crnobarac, J., Jaüimović, G., Marinković, B., Mircov, V. D., Mrda, J., & Babić, M. (2009). Dynamics of pot marigold yield formation depended by varieties and row distance. *Natural Products Communication*, *4*(1), 35–38. doi: 10.15835/hpm.v16i1-2.3040.

Defforey, D., & Paytan, A. (2018). *Global Phosphorus Dynamics*. https://doi.org/10.1093/OBO/9780199363445-0097.

Dogramacı, M., & Arabacı, M. (2010). The Effect of the Organic and Inorganic Fertilizer Applications on Yield and Yield Components of Anise (*Pimpinella anisum* L.) Type and Ecotypes. *Journal of Adnan Menderes Unıversıty Agrıcultural Faculty*, *7*(2), 103–109.

Egeli, D. (2022). *Calendula officinalis* L.. In: Guragac Dereli, F.T., Ilhan, M., Belwal, T. (eds) Novel Drug Targets With Traditional Herbal Medicines. Springer, Cham. https://doi.org/10.1007/978-3-031-07753-1_4

Ganjali, H. R., Band, A. A., Sharif Abad, H. H., & Moussavi, N. M. (2010). Effects of sowing date, plant density and nitrogen fertilizer on yield, yield components and various traits of *Calendula officinalis*. *American-Eurasian Journal of Agricultural & Environmental Sciences*, *6*, 672–679.

Gupta, N., Gupta, A. K., Gaur, V. S., & Kumar, A. (2012). Relationship of nitrogen use efficiency with the activities of enzymes involved in nitrogen uptake and assimilation of finger millet genotypes grown under different nitrogen inputs. *The Scientific World Journal*.

Hao, D., Luan, Y., Wang, Y., & Xiao, P. (2024). Unveiling Nitrogen Fertilizer in Medicinal Plant Cultivation. *Agronomy*. https://doi.org/10.3390/agronomy14081647.

Hong, N., Scharf, P. C., Davis, J. G., Kitchen, N. R., & Sudduth, K. A. (2007). Economically optimal nitrogen rate reduces soil residual nitrate. *Journal of Environmental Quality*, *36*, 354–362. https://doi.org/10.2134/jeq2006.0173

Hussain, I., Ullah, R., Khan, N., Ayaz, S., Ahmad, S., Shanzeb, Ahmed, M., Hasan, P. T., & Khan, F. A. (2011). Phytochemicals and inorganic profile of *Calendula officinale* and *Sonchus asper*. *African Journal of Pharmacy and Pharmacology*, *5*(16), 1813–1818.

Jane, M. F., Johnson, R. W., Russ, W. G., & Barbour, N. W. (2018). Limited seed and seed yield response of *Calendula* to applied nitrogen does not justify risk of environmental damage from high urea application rates. *Agriculture*. https://doi.org/10.3390/agriculture8030040

Jevdovıc, R., Todorovıc, G., Kostıc, M., Protıc, R., Lekıc, S., Zıvanovıc, T., & Secanskı, M. (2013). The effects of location and the application of different mineral fertilizers on seed yield and quality of pot marigold (*Calendula officinalis* L.). *Turkish Journal of Field Crops*, *18*(1), 1-7.

Kacar, B. (2020). *Sürdürülebilir tarımda insan ve hayvanlarda fosfor*. Nobel Yayın No: 2588, Ankara. (in Turkish)

Kareem, A., Saeed, S., & Hammad, H. M. (2014). Growth and performance of *Calendula officinalis* L. on different crop residues. *World Journal of Agricultural Sciences*, *2*(5), 98–101.

Król, B. (2011). Yield and the chemical composition of flower heads of pot marigold (*Calendula officinalis* L. cv. Orange King) depending on nitrogen fertilization. *Acta Scientiarum Polonorum Hortorum Cultus*, *10*(2), 235–243.

Krol, B. (2017). Influence of nitrogen fertilization on the seed yield and the content and quality of fat in pot marigold (*Calendula officinalis* L.) cultivars. *Agronomy Science*, *72*(3), 85–98.

Kumar, A., & Singh, A. K. (2011). Effect of spacing and nitrogen levels on vegetative growth, flowering behavior, and yield of calendula (*Calendula officinalis* L.). *Plant Archives*, *11*(2), 941–944.

Kumar, V., Pandey, S. K., Singh, V. K., Verty, P., & Samoon, S. A. (2015). Response of nitrogen and phosphorus levels on calendula (*Calendula officinalis* L.). *Research in Environment and Life Sciences, 8*(4), 557–560.

Kwiatkowski, C. A., Harasim, E., & Yakimovich, A. (2020). Effect of nitrogen fertilization and plant density on seed yield and fat content and quality of pot marigold (*Calendula officinalis* L.) under climatic conditions of Belarus. *Acta Scientiarum Polonorum Hortorum Cultus, 19*(5), 3–12. DOI: 10.24326/asphc.2020.5.1

Kyveryga, P. M., Blackmer, T. M., & Caragea, P. C. (2011). Categorical analysis of spatial variability in economic yield response of corn to nitrogen fertilization. *Agronomy Journal, 103*, 796–804. https://doi.org/10.2134/agronj2010.0411

Marek, D., Michał, M., & Cezary, P. (2018). Source of nitrogen as a factor limiting saponin production by hairy root and suspension cultures of *Calendula officinalis* L. *Acta Physiologiae Plantarum*. https://doi.org/10.1007/S11738-018-2610-2

Meisinger, J. J., & Delgado, J. A. (2002). Principles for managing nitrogen leaching. *Journal of Soil and Water Conservation, 57*(6), 485–498. https://doi.org/10.1080/00224561.2002.12457483

Moll, R. H., Kamprath, E. J., & Jackson, W. A. (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agronomy Journal*, *74*(3), 562–564. https://doi.org/10.2134/agronj1982.00021962007400030037x

Mollafilabi, A., Khorramdel, S., Siahmarguee, A., & Shoorideh, H. (2014). Plant density and nitrogen fertilizer effects on yield and qualitative characteristics of marigold (*Calendula officinalis* L.) under Torbat-e-Jam climatic conditions. *International Journal of Advanced Biological and Biomedical Research, 2*(4), 1201–1209.

Morris, TF., Murrell, TS., Beegle, DB., Camberato, JJ., Ferguson, RB., Grove J., Ketterings, Q., Kyveryga, PM., Laboski, CAM., McGrath, JM., Meisinger, JJ., Melkonian, J., Moebius-Clune BN., Nafziger, ED., Osmond, D., Sawyer, JE., Scharf, PC., Smith, W., Spargo., JT, van Es HM. & Yang, H., (2018), Strengths and limitations of nitrogen rate recommendations for corn and opportunities for improvement, *Agron J* , 110:1-37pp. https://doi.org/10.2134/agronj2017.02.0112.

Mousavi, S. S., & Shahraki, H. R. (2012). Effect of nitrogen and phosphorus on essential oil yield and chemical composition of *Calendula officinalis* L. *International Journal of Agriculture and Crop Sciences, 4*(8), 491–497.

Nagmote, R. S., Kuchanwar, O. D., Chopde, N. K., Kausadikar, P. H., Gopal, K. R. & Panghate, P. A., (2020), Effect of graded level of nitrogen and phosphorus on yield, quality and total uptake of *Calendula*, *IJCS*, *8*(2): 1680-1682pp. DOI: 10.22271/chemi.2020.v8.i2z.9003

Nematpour, A., Eshghizadeh, H. R., Zahedi, M., & Gheysari, M. (2020). Interactive effects of sowing date and nitrogen fertilizer on water and nitrogen use efficiency in millet cultivars under drought stress. *Journal of Plant Nutrition*, *43*(1), 122-137. https://doi.org/10.1080/01904167.2019.1659351.

Novoa, R. & Loomis RS., (1981), Nitrogen and plant production, Plant Soil 58:177-204pp.

Odoom, A., & Ofosu, W. (2024). *Role of Phosphorus in the Photosynthetic Dark Phase Biochemical Pathways*. IntechOpen. https://doi.org/10.5772/intechopen.112573

Pandey, R.K. & Mishra, A., (2005), Effect of nitrogen, phosphorus and potassium on growth, flowering and seed yield in marigold cv. Pusa Narangi Gainda. *Progressive Horticulture*, 37(2): 341-344pp.

Pazoki, A., Tavakoli Haghighat, H. & Rashidi Asl, A., (2016). Evaluation of yield, yield components and essential oil content of marigold (*Calendula officinalis* L.) with the use of nitrogen and vermicompost. *Journal of Crop Ecophysiology*, *10* (3): 629-644pp.

Rahemi, M., (2004). Pollination and fruit formation. Publication of Shiraz University.

Rahmani, N., Daneshian, J., Farahani, H. A. & Taherkhani, T., (2011). Evaluation of nitrogenous fertilizer influence on oil variations of Calendula (*Calendula officinalis* L.) under drought stress conditions, *Journal of Medicinal Plants Research*, *5*(5): 696-701pp.

Rahmani. N., Aliabadi, F.H., & Valadabadi, S.A.R. (2008). Effect of nitrogen on oil yields and its components of calendula (*Calendula officinalis* L.) in drought stress conditions, *Abstracts Book of World Congress on Medicinal and Aromatic Plants*, South Africa, 364p.

Rawal, N., Pande, K. R., Shrestha, R., &Vista, S. P. (2022). Nutrient use efficiency (NUE) of wheat (*Triticum aestivum* L.) as affected by NPK fertilization. *Plos one*, *17*(1), e0262771. https://doi.org/10.1371/journal.pone.0262771

Rupp, H., Tauchnitz, N., & Meissner, R. (2021). The effects of soil drying out and rewetting on nitrogen and carbon leaching–results of a longterm lysimeter experiment. *Water*, *13*(18), 2601. https://doi.org/10.3390/w13182601

Samoon, S. A., Bashir, Z., Kirad, K. S. & Lone, A. A. (2018). Effect of nitrogen and phosphorus levels on flowering of calendula (*Calendula officinalis* L) var. touch of red Mixture. The Pharma Innovation Journal, *7*(5, Part A), 39.

SAS Institute Inc. (2018). JMP statistical software, Version 14. Cary, USA.

Scaini, A., Vico, G., Thorslund, J., Hugelius, G. & Manzoni, S. (2023). Water Availability and Land Management Control Catchment-Scale Agricultural Nitrogen and Phosphorous Use Efficiencies. *Global Biogeochemical Cycles*, *37*(1), e2022GB007487. https://doi.org/10.1029/2022GB007487

Serra, A. P., Marchetti, M. E., Vieira, M. C., Robaina, A. D., Veronesi, C. O., Nascimento, J. M., & Guimarães, F. C. N. (2013). Nitrogen nutritional efficiency and biomass production in *Calendula officinalis* L. (Asteraceae) under greenhouse conditions. *Revista Brasileira de Plantas Medicinais, 15*, 78–85. https://doi.org/10.1590/S1516-05722013000100011

Shil, S. & Dewanjee, S. (2022). Impact of drought stress signals on growth and secondary metabolites (SMs) in medicinal plants. *J Phytopharmacol*, *11*(5), 371-376. doi:10.31254/phyto.2022.11511

Statham, P. J. (2012). Nutrients in estuaries-An overview and the potential impacts of climate change. *Science of the total environment*, *434*, 213-227. doi: 10.1016/j.scitotenv.2011.09.088. Epub 2011 Nov 25. PMID: 22119025.

Ullah, H., Santiago-Arenas, R., Ferdous, Z., Attia, A., & Datta, A. (2019). Improving water use efficiency, nitrogen use efficiency, and radiation use efficiency in field crops under drought stress: A review. In *Advances in agronomy* (Vol. 156, 1st ed.). Elsevier Inc. https://doi.org/10.1016/bs.agron.2019.02.002

Verma, A. K., Yadav, B., Kumawat, C., & Yadav, P. (2017). Rhizosphere Management Strategies for Enhance Phosphorus Utilization by Crops: An Overview. *International Journal of Current Microbiology and Applied Sciences*, *6*(4), 446–452. https://doi.org/10.20546/IJCMAS.2017.604.051.

Wang, B., Liu, W., & Dang, T. (2011). Effects of phosphorus on crop water and nitrogen use efficiency under different precipitaion year in dryland. 3:2111-2114. doi: 10.1109/ISWREP.2011.5893679.

Wang, X., Zhang, C., Zhao, N., Sun, X., Hou, S., & Wang, P. (2024). Physiological Nitrogen Uptake and Utilisation Responses in Two Native Plants from the Qinghai-Tibet Plateau under Different Water and Fertiliser Conditions. Agronomy, 14(3), 440. https://doi.org/10.3390/agronomy14030440.

Webb, C.J., Sykes, W.R. & Garnock-Jones, P.J. (1988). Flora of New Zealand. Vol. IV. Naturalised Pteridophytes, Gymnosperms, Dicotyledons. Botany Division DSIR, Christchurch.

Wickham, H., M. Averick, J. Bryan, W. Chang, L.D.A. McGowan, R. François, & H. Yutani. (2019). Welcome to the Tidyverse. Journal of Open-Source Software 4(43):1686. doi: 10.21105/joss.01686.

Wickham, H., W. Chang, & M.H. Wickham. (2016). Package 'ggplot2'. Create elegant data visualisations using the grammar of graphics. Version 2(1):1-189. https://doi.org/10.1007/978-3-319-24277-4

Xue, B. L., Guo, Q., Otto, A., Xiao, J., Tao, S. & Li, L. (2015). Global patterns, trends, and drivers of water use efficiency from 2000 to 2013. *Ecosphere*, *6*(10), 1-18. https://doi.org/10.1890/ES14-00416.1

Ye, J. Y., Tian, W. H., & Jin, C. W. (2022). Nitrogen in plants: from nutrition to the modulation of abiotic stress adaptation. Stress Biology, 2(1), 1–14. https://doi.org/10.1007/s44154-021-00030-1.

Yetis, C. (2019). The effects of plant density on flower yield and some active components of pot marigold (*Calendula officinalis* L.). Master Thesis, Ondokuz Mayis University. 74p.

Zhang, J. (2024). Effects of Water and Nitrogen on Eco-physiological Characteristics of Two Dominant Grassland Species on the Semiarid Loess Plateau. Science and Technology of Engineering, Chemistry and Environmental Protection, 1(10). https://doi.org/10.61173/606q2z36