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Effects of Germination Optimization and Fertilizer Strategies on Drought Resilience and Oil Productivity in Sunflower

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1. RESEARCH BACKGROUND AND OBJECTIVES

Sunflower (*Helianthus annuus* L.) is an important oilseed crop worldwide. Sunflower seeds offer multiple advantages, including high oil and protein content. Sunflower oil is primarily utilized for human consumption and biodiesel production. It is a cash crop that has gained economic importance in many countries, among them Hungary is considered as leading country in sunflower production. The country's suitability for sunflower farming arises from the plant's ability to flourish in hot summers and well-drained soils, making it an appropriate option in the face of climate change challenges. Despite its drought-tolerant nature compared to other crops, sunflower cultivation faces challenges, especially in arid conditions and elevated temperatures during critical growth stages from germination to the filling period. Seed germination is a critical stage in crop production that significantly impacts nutritional value and yield. The importance of this phase lies in its role in establishing a robust foundation for plant development, enabling seedlings to grow strong roots and efficiently absorb nutrients. The success of seed germination is crucial for developing healthy crops, directly affecting plant growth, resilience, and productivity. Moreover, effective germination reduces weed competition and susceptibility to pests. Adequate water and temperature are crucial factors for seed germination. It is essential to understand how crop seeds respond to temperature during germination and emergence, as this knowledge identifies their tolerance to low and high temperatures and the climatic conditions in which partial crops can successfully germinate and establish. Drought conditions exacerbate this issue, significantly affecting the critical germination period. Improved moisture retention helps mitigate drought effects by ensuring a more stable water supply to plants. This is especially important for sunflower cultivation, as the crop is particularly vulnerable to drought and heat stress following germination, most critically during the early flowering and achene filling stages. Insufficient rainfall and low soil moisture lead to reduced yields due to poor regulation of leaf expansion and transpiration. Optimizing germination alone is insufficient to ensure drought resilience and successful growth in sunflowers. Throughout their developmental stages, from plant growth to flowering and seed maturation, sunflowers heavily depend on the availability of water and fertilizers to achieve optimal development. In sunflower cultivation, fertilizer practices play a vital role in boosting growth, yield, and oil content. Fertilizers supply essential nutrients for plant development, facilitating carbohydrate assimilation, protein synthesis, and the production of crucial photo-assimilates and by-products necessary for seed formation. Fertilizer applications can deliver vital nutrients to plants during favorable water conditions or periods of drought. Moreover, organic fertilizers enhance soil structure by increasing organic matter, which improves moisture retention. Integrated Nutrient Management (INM) is an agricultural approach that reduces reliance on chemical fertilizers by combining organic and inorganic nutrient sources, resulting in improved crop growth and yields. It enhances nutrient efficiency and balanced fertilization, aligning with crop needs and soil availability, optimizes crop yields by improving both nutrient availability and soil fertility, and improves soil microbial biomass, which supports nutrient cycling and plant health. Additionally, INM contributes to sustainable agriculture by improving soil health, enhancing nutrient uptake efficiency, reducing environmental risks, and lowering fertilizer costs, while also helping to decrease greenhouse gas emissions. This practice is gaining popularity as an environmentally friendly alternative to conventional farming methods. This approach improves growth and yield in various crops, including sunflowers. In the literature reviews, the manure or compost combined with recommended fertilizers or single nitrogen with or without biofertilizers was investigated for their effects on sunflowers yield and oil production, and provided significant results. Despite the extensive research available, a significant gap in knowledge persists regarding the combined use of potassium and Effective Microorganisms (EM), especially in sunflowers. While the individual roles of potassium in physiological processes and the biological advantages of EM have been explored separately, their synergistic effects have not been adequately examined. Additionally, combinations that include organic nitrogen (from cattle manure), inorganic nitrogen, and EM, as well as the combination of organic nitrogen, inorganic nitrogen, and potassium, have not been thoroughly studied to sunflower yield and oil quality. This indicates a new and relatively unexamined path for improving nutrient use efficiency and enhancing the sustainability of oilseed cultivation. Additionally, the determination of optimal temperature, water requirements, seed number per Petri dish, and methods for managing fungal growth during sunflower seed germination under controlled conditions has been scarcely investigated.

This study aims to:

- Optimize germination conditions (temperature, water, seed number, fungal growth) for sunflower seeds under in vitro conditions.
- Investigate the effects of organic and inorganic fertilizers on sunflowers' growth and oil quality over three consecutive years.

- Determine the appropriate integrated fertilization management for sunflower production

This research aims to achieve a comprehensive approach to improving sunflower productivity and resilience by optimizing both the germination phase and fertilization practices.

2. MATERIALS AND METHODS

The research study is divided into two sections: the first section emphasizes the optimization of seed germination *in vitro*, while the second section investigates the integrated fertilizer practice of sunflowers in the field under the conditions of rainfed agriculture. The methodology of germination experiments was originally developed in our department in wheat and maize. In 2022, we conducted an *in vitro* assessment of seed germination and seedling growth in a variety of contexts. These variables encompassed a variety of temperatures, water levels seed quantities per Petri dish, and antifungal methods. The seed germination test was conducted in 2024. For all experiments, we used the sunflower hybrid "ES Emeric", a mid-early oleic variety that received registration in Italy in 2020. This hybrid is tolerant to Imazamox and shows moderate susceptibility to Verticillium, Phomopsis, and Sclerotinia capitulum. The seeds, treated with Fludioxonil + Metalaxyl, had a thousand kernel weight of approximately 101.4 g. For the field experiment, the methodology of this experiment was developed based on the literature review showing how the fertilizers Potassium, Nitrogen (50% Organic + 50% inorganic), and biofertilizers (effective microorganisms) and their synergies contribute to increasing resilience to drought and productivity of sunflower crop under rainfed conditions. From 2022 to 2024, we implemented a three-year field experiment to assess the efficacy of various fertilizer methods on sunflower crops that were cultivated in rainfed conditions. The objective of this investigation was to determine the most advantageous approach for producers in scenarios where water resources are scarce.

2.1.Laboratory Protocols for Germination Studies

The experiment was carried out on sunflower seeds (*Helianthus annus L.*) at the Institute of Agronomy (Hungarian University of Agriculture and Life Sciences).

In 2022, we explored the effects of abiotic stress factors (water availability and temperature), seedling density, and fungal growth control on seed germination and seedling development. The 2024 experiment focused specifically on assessing the germination rate of sunflower seeds at various temperatures. All experiments were conducted in vitro using a growth or climate chamber (ICO105, Memmert GmbH + Co. KG, Schwabach, Germany).

Germination tests

The initiation of seed germination in Petri dishes containing filter paper was investigated at varying temperatures during this phase of the experiment. The temperatures that were recorded were 5, 10, 15, 20, 25, 30, 35, and 40 degrees Celsius. This experiment employed five replications per treatment, with 10 seeds per Petri Dish (PD) and 9 ml of distilled water added in each case. For 14 days, the number of germinated seedlings was counted daily at each temperature.

Temperature tests

Germination was evaluated at eight constant temperatures—5 ◦C, 10 ◦C, 15 ◦C, 20 ◦C, 25 ◦C, 30 ◦C, 35 ◦C, and 40 ◦C—using a growth chamber. Ten sunflower seeds were placed in a normal 9 cm Petri dish with tissue paper, and 9 mL of distilled water was added. The germination of a seed was recorded when the radicle emerged, and the length of the seedlings in the Petri dishes was measured after around 80% of them had reached 1 cm in length. On a daily basis, four PD were removed from each temperature chamber in order to physically measure the length of the radicles and shoots; four replications per treatment were used in this experiment.

Water amount tests

The germination response of sunflower seeds to water stress was examined under 30 different water levels. Twelve different amounts of distilled water were used based on a milliliter interval from 0 to 10, and 18 different amounts of distilled water were the basis of the TKW as a percentage. Fifty seeds were placed in five PD at each water level; five replications were used for this experiment. Then, the seeds were incubated at 20 ◦C for ten days in a growth chamber. The following Equation was used to compute the amount of water based on TKW:

$$\text{TKW} * \text{Seed number}/100{,}000 = 1\% \text{ of the proposed water amount}$$

The TKW of the sunflower seeds was 50.46 g. The PD was sealed with parafilm to prevent water evaporation, and a growth chamber was used to incubate the PD at 20°C. After 10 days of incubation, the lengths of the radicles and shoots, in addition to the number of non-germinated seeds, were measured. In addition, the radicles and shoots were dried at 65°C until they acquired a constant weight after being separated (48 h).

Seed number tests

This part of the experiment examined the effect of seed numbers on germination and seedling development using the same volume of water (9 mL) in PD. Four sets of seeds were used—6, 8, 10, and 12—and they were incubated in a growth chamber at 20°C. After ten days, the radicle and shoot lengths and the number of non-germinated seeds were measured. This experiment was repeated ten times. The radicle and shoot were then separated and dried at 65 °C until they attained a consistent weight (48 h).

Antifungal Control tests: The efficiency of the fungicide in inhibiting fungal growth was evaluated using two alternative antifungal application methods. The first method: the growth media were treated with five different concentrations of Amistar Xtra in the first method: 0, 20, 200, 2000, and 20,000 ppm. The second method: two different seed sterilization techniques were examined: the first consisted of soaking the seeds in a 2000 ppm Amistar Xtra solution for three minutes, and the second involved 10% sodium hypochlorite (NaClO) with the same method. The seeds were sterilized, rinsed with distilled water, and then incubated in the growth chamber for 10 days at 25°C. After ten days of incubation, the radicle and shoot lengths were measured, together with the number of seeds that had germinated. This experiment was performed ten times.

2.2.Field Production Trials

The present study was conducted in the experimental field of the Hungarian University and Life Sciences (MATE) in Gödöllö, Hungary, for three years 2022,2023, and 2024. The experimental site in Gödöllö is located at the latitude 47°35'41" N and longitude 19°22'07"E. The experimental field used sand-based brown forest soil (Chromic Luvisol), with sandy loam texture and variable clay content. The soil was susceptible to compaction, poor water retention, and drought impacts. The Meteorological data was collected during three years of the experiment from 2022 to 2024.

Soil preparation: Soil preparation involved plowing, harrowing, and seedbed preparation using a moldboard plow, ensuring a fine seedbed for optimal sunflower root development and water infiltration.

Planting: The sunflower was sown on 5th May, 25th April, and 8th May respectively in 2022,2023, and 2024. Seeds were sown directly into the seedbed, spaced 75 cm apart, and timed to coincide with soil temperatures above 15°C.

Plant protection: Weed control was applied to sunflower plants before planting, and seeds were covered to prevent seed loss due to avian consumption over three years.

Details of treatments: This study was carried out by implementing a total of 7 distinct treatments including control plants, each of which was meticulously designed to represent types of fertilizers (organic, inorganic, and biofertilizers). We used the Nitrogen split from Dried pelleted cattle manure and Ammonium Nitrate, potassium K from Sulphate of Potash and biofertilizer as effective microorganism containing photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes, and fermenting fungi (Higa, 1991). The dried pelleted cattle manure - an organic fertilizer produced from composted, dehydrated cow manure was selected for its soil-structure improvement and gradual nutrient release properties. We applied them individually and in combination to test their synergies potential in increasing drought resilience of productivity of Sunflower crops. Each treatment was replicated three times, thereby increasing the robustness and accuracy of the findings. The treatments were applied at the vegetative stage (BBCH 14).

- **CONTROL**: Control plants
- **POTASSIUM**: recommended Potassium K (100 kg/ha of Sulphate of Potash)
- **GOIM**: recommended Nitrogen N (100 kg/ha) from 50% organic manure (manure of cattle) and 50% inorganic manure (Ammonium nitrate)
- **EM-1:** Effective micro-organismes 40 l/ha (EM1)
- **K+GOIM**: combination of K and GOIM
- **K+EM-1**: combination of K and EM-1**.**
- **GOIM+EM:** combination of GOIM and EM

Details of the collection of experimental data: Three plants from each plot were selected at random and tagged to record various observations. Observations on growth parameters and yield components were recorded at three distinct stages of crop growth. The growth parameters, such as SPAD output, Leaf Area Index, Plant height, leaf number, stem girth, or stem diameter, Chlorophyll Fluorescence, and Head diameter, were measured in the field. The yield parameters, such as the Number of seeds per head, TKW, were recorded in the laboratory of the Institute of Agronomy at the Hungarian University of Agriculture and Life Sciences. The quality parameters (oil content, protein, water content) and Fatty acid profile were determined in MATE Központi Vizsgálólaboratórium in Kaposvár. Concerning harvesting and threshing, the crop was harvested between the end of September and the beginning of October each year. The head of the crop was harvested first, followed by cutting the stalks with sickles. Later, the heads were sun-dried for about a week and threshed to separate the seeds from the heads. The seeds were dried, cleaned, and weighed to determine the yield per hectare. Furthermore, the study evaluated sunflower yield attributes to assess productivity and quality under different fertilizer levels. Key parameters included head diameter, head weight, seed number per head, Thousand Kernel Weight, seed yield, quality parameters (oil content, seed moisture content, protein percentage), and fatty acid profile. The results provide valuable insights into sunflower plant growth and productivity.

Statistical analysis

For the germination experiment, a sigmoid curve model was used to fit the data and plot temperature levels. ANOVA and LSD were used for water, seed number and fungal growth experiments. Germination indices were calculated, and fitting curves were created using R packages. For the field experiment, we used IBM SPSS V27 and RStudio V2024.04.2 software, along with R packages, to analyze the effects of different fertilizer treatments and two years on sunflower plant growth and yield attributes. Heatmaps clustering and AMMI PCA were used to visualize interaction patterns between treatments and explore the relationship between fertilizer types and year factors. Data normality verifications were performed using Kolmogorov–Smirnov and Shapiro–Wilk tests.

3. RESULTS AND DISCUSSION

This chapter highlights the results of sunflower seed germination experiments under controlled conditions, followed by an analysis of the field trials, examining the impact of various fertilizer treatments on sunflower growth, physiology, yield, and oil content over three seasons.

3.1. Experimental research: Germination and Seedling Development Responses of Sunflower seeds to different temperatures, water levels, seed number, and fungal growth methods under controlled conditions

This study examines the optimal conditions of seed germination under different levels of temperature, water, seed number, and different methods to control fungal growth in vitro.

3.1.1. Germination test

The study reveals that sunflower seeds' germination performance varies significantly across temperature ranges from 5°C to 40°C (Figure 1). At 5°C, germination is slow and delayed, with a maximum rate of 13.33%. The mean germination time is 8.98 days, and the area under the curve is low. The time to reach 50% germination is 12.96 days. At 10°C, germination improves significantly, with a maximum rate of 53% and quicker germination. However, 10°C remains below the optimal temperature for sunflower germination. At 15°C, sunflower seeds showed a 75% germination rate, indicating efficient physiological processes. The time to maximum germination (TMGR) was 3.52 days, indicating a significant acceleration in germination speed. The AUC was 784.219, and the shorter MGT (4.12 days) and T50 (3.52 days) suggest 15°C is optimal for maximizing germination rate. At 20°C, the germination rate decreased slightly to 66%, but other parameters continued to improve. At 25°C, the rate dropped further to 55.55%, with shorter MGT and AUC. The optimal temperature range for sunflower germination is between 15°C and 30°C. At 30°C, the germination process is highly efficient and consistent, allowing for rapid seedling establishment. At 35°C, the germination rate is high but may induce physiological stress, limiting the success. At 40°C, the maximum germination rate declines to 24%, and the AUC drops to 307.62. The AUC (Area Under the Curve) is highest at 30°C, suggesting that this temperature is particularly effective for rapid and successful germination. Significant germination occurs at the optimal temperature range for sunflower seed germination, being 15°C to 30°C. The optimal germination percentage, germination timing, and seedling growth are guaranteed within this range. The germination process is slowed by cold stress and reduced metabolic activity at low temperatures of 5°C and 10°C, whereas the reduced germination rates at 35°C and 40°C can be attributed to heat stress and physiological damage. The seeds' capacity to accomplish optimal germination is restricted by these extreme temperatures, which are both cold and hot. Sunflower seeds are more productive within the 15°C to 30°C temperature range.

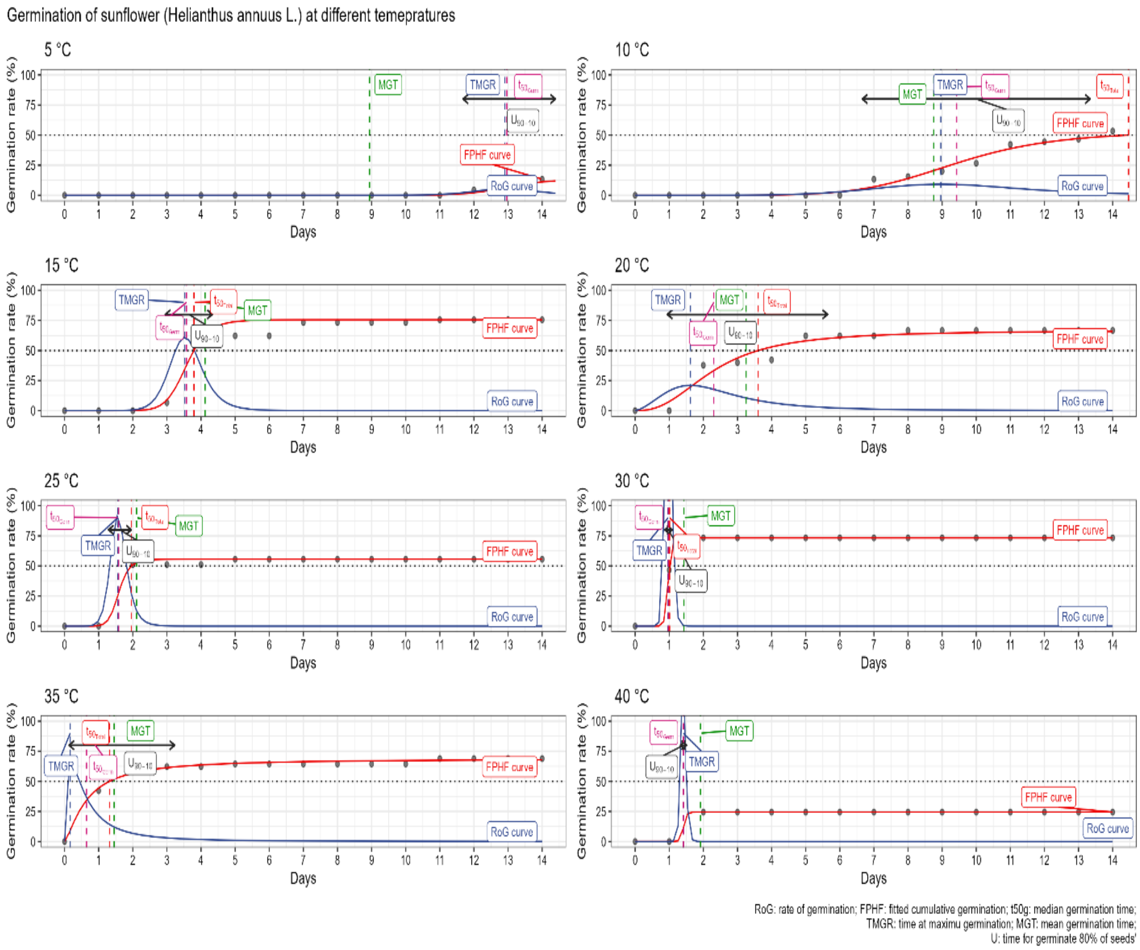

Figure 1. Germination rate at different temperature levels

3.1.2. Effect of Temperature on seed germination and seedling growth

The study examined the germination of sunflower seeds at various temperatures, with the highest seedling growth rate observed at 25°C. Early seedling growth occurred at 20°C and 25°C, but at 20°C, the growth rate was lower. Low temperatures took approximately 19 days to germinate, with the minimum temperature being 5-10°C and the maximum between 35-40°C. The study found that the highest growth rate was at 25°C, followed by 15°C, 20°C, and 35°C.

Temperatures above the ideal range were found to be detrimental to seedling development, with lowered length values at 35°C and the absence of germination at 40°C.

3.1.3. Effect of water on seed germination and seedling growth

The growth of seedlings increased significantly with water volume to the optimal level but decreased significantly as water quantity increased beyond the optimal level. The optimal water range for seedling length was 8,2–11,4, representing 1625–2250 percent of TKW. Moreover, it was within the ideal range indicated by the one milliliter (8–12 ml) water-based approach. Therefore, it can be stated that the TKW approach is more precise in optimizing germination water requirements. The optimal range of dry matter content for the seedling was evaluated to be 4.4–8.4 ml, corresponding to 850–1750% of TKW . As a result, the seedling values' dry weight decreased at a level greater than 8.8 ml.

3.1.4. Effect of seed density on seed germination and seedling growth

The results indicated a non-significant difference in the seedling length and a significant difference in dry seedling weight among aggregated values of 6, 8, 10, and 12 seeds per PD used for the seed number test. Furthermore, all the parameters measured significantly increased as the number of seeds increased. Therefore, seed densities of 10–12 appeared ideal for developing a sunflower crop in vitro

3.1.5. Effect of antifungal methods on seed germination and seedling growth

Seedling growth declined proportionally with increasing antifungal concentrations, even at low doses. The fungicide Amistar Xtra adversely affected seedling development, consistent with its primary mode of action as a fungal growth inhibitor. These results demonstrate that this product should not be applied directly to seeds and is best suited for foliar application in the field, which aligns with its intended use. Compared to the treated seeds in the growing medium with Aamistar Xtra, the priming approach with 10% Sodium hypochlorite (N_aClO) enhanced all growth indicators significantly. Priming or pre-treating seeds can be a useful option for reducing fungal growth during in vitro seed germination.

4. Experimental Research: Effects of integrated fertilizer methods on sunflower growth, physiological responses, and oil quality over the years

This study examines the growth parameters (leaf number, stem diameter), physiological responses (leaf area index LAI, SPAD chlorophyll output), yield attributes (head diameter, weight of diameter, seed number per head, Thousand Kernel Weight, yield) and oil quality characteristics (moisture content, oil content, protein content, and fatty acid composition) of sunflower in response to fertilizer methods between 2022 and 2024.

4.1. Sunflower growth and physiological responses to fertilizer treatments over the years

The growth and physiology of sunflower crops were enhanced through the integrated use of organic, inorganic, and biofertilizers. The study found significant differences in sunflower growth and physiology parameters between treatment and year factors, with the Leaf Area Index (LAI) showing a significant year effect, fertilizer effect, and year × fertilizer treatment interaction. All treatments had significantly higher LAI in 2023, followed by 2024 and 2022. The K+GOIM treatment had the highest LAI levels in 2022, with the lowest values in GOIM+EM and K+EM. The notable improvement in LAI seen in the K+GOIM treatment indicates that this fertilizer combination maximizes canopy growth, leading to an increased leaf surface area that can absorb more light for photosynthesis. LAI is an essential indicator of a plant's capacity to absorb sunlight, which directly affects its photosynthetic efficiency and, consequently, biomass generation. The number of leaves in sunflowers is crucial for growth, as it affects photosynthesis, biomass production, and yield potential. A study found a significant year effect, fertilizer effect, and year × fertilizer treatment interaction on leaf production. The number of leaves increased significantly in 2023, followed by 2024, and 2022. In 2022, K+GOIM generated the most significant values (19 green leaves), followed by EM (11 green leaves). The leaf number in control plants was about 7. In 2023 and 2024, the values of K+GOIM and EM were marginally lower than those of the other treatments, but no significant difference was observed for the other years. SPAD readings reflect chlorophyll content in sunflower leaves, which directly affects photosynthesis, biomass growth, and final yield**.** Additionally, the study reveals that the year, fertilizer, and year × fertilizer treatment interaction significantly affect the SPAD output. The most significant treatments in 2022 were K+GOIM and EM, with no significant disparity observed in other years. The improved chlorophyll content is further supported by the increased leaf number and SPAD values seen in these treatments, which are associated with improved photosynthetic efficiency and general plant health. The K+GOIM and EM treatments showed higher leaf numbers and SPAD values, which indicate improved chlorophyll content and general plant health. Increased chlorophyll content, shown by higher SPAD values, is strongly linked to better photosynthetic efficiency, which supports more robust plant development.

4.2.Responses of sunflower yield characteristics to fertilizer treatments across years

The present study identified significant differences in sunflower yield parameters across both treatment and year factors, with a marked treatment effect and a significant interaction between year and treatment. Among the yield components, sunflower head weight emerged as a reliable metric for assessing both seed production and oil yield potential. Notably, the highest head weight was recorded in 2023, followed by 2024 and 2022, respectively (Figure 2). In 2022, the combination of potassium, organic nitrogen, and inorganic nitrogen (K+GOIM) significantly increased head weight, whereas in 2023, the greatest head weights were observed under the GOIM+EM, EM, and K treatments. Similarly, in 2024, the K+EM and GOIM treatments achieved the highest head weight values. Building upon the findings related to head weight, sunflower head diameter was also found to play a crucial role in determining the seed-forming capacity, as larger heads typically produce more seeds, thereby contributing to higher overall yields. Fertilization treatments and interannual variability significantly influenced head diameter, with an approximately 50% increase observed in 2023 compared to 2022 (Figure 2). Particularly in 2022, the combined application of potassium, organic nitrogen, and inorganic nitrogen (K+GOIM) notably enhanced head diameter. Further post-hoc analysis revealed that the GOIM+EM treatment resulted in the largest head diameters in 2023, reinforcing the importance of integrated fertilization strategies. In a similar trend, the study revealed a significant increase in sunflower seed weight from 2022 to 2024, with the most substantial increase recorded in 2023 (Figure 2). Treatment effects were especially pronounced in 2022 and 2024. Specifically, the K+GOIM treatment yielded the highest seed weight values in 2022, whereas the GOIM+EM treatment achieved the maximum seed weight among all fertilizer treatments in 2023. These results emphasize the critical role of targeted nutrient management in enhancing seed development under varying environmental conditions. The Thousand Kernel Weight (TKW), another key yield determinant, was significantly influenced by year, treatment, and their interaction (Figure 2). In 2022, the K+GOIM treatment recorded the highest TKW values. During 2023, the treatments GOIM+EM, EM, and K+EM produced the most significant TKW values, indicating the beneficial effects of combining organic, inorganic, and biological inputs. A similar pattern was observed in 2024, suggesting consistent treatment efficacy over time. Furthermore, the K+GOIM treatment consistently produced the highest yields in 2022 and 2024, while in 2023, the K and GOIM treatments showed superior performance. It is noteworthy that 2022, the driest year during the experimental period, still recorded the highest yields under the K+GOIM treatment. This finding underscores the synergistic interaction between potassium and combined organic and inorganic nitrogen sources (K+GOIM), which appears to create optimal growth conditions even under moisture-limited environments in 2022. Enhanced head weight, head diameter, seed weight, and thousand-kernel weight (TKW) were key contributors to the observed yield improvements. In addition, the GOIM+EM treatment, which incorporates beneficial microorganisms, demonstrated outstanding yield characteristics, particularly in 2023. This suggests that environmental conditions in 2023, likely including favorable soil moisture and enhanced microbial activity, may have amplified the effectiveness of the integration between organic and inorganic materials with microbial inoculants. These results highlight the potential for effective microorganisms (EM) to further enhance sunflower yield parameters under certain environmental conditions, offering an additional strategy for sustainable yield improvement.

4.3.Effects of fertilizer treatments and year on seed quality over the years

Sunflower seed quality, encompassing oil content, protein composition, and moisture levels, is profoundly influenced by agronomic practices and environmental variability, making it a critical focus for optimizing both nutritional and industrial value. The present study revealed significant differences in sunflower seed quality parameters across both fertilizer treatments and years, with oil content emerging as a key factor influencing overall seed quality and yield potential (Figure 3). Notably, the main effects of year and fertilizer treatment on oil content were highly significant. In 2022, the K and K+EM treatments achieved the highest oil contents, reaching 48%, while in 2023, the K+GOIM and GOIM+EM treatments produced comparably high values of approximately 43%. These findings suggest that potassium application, particularly when combined with effective microorganisms, plays a pivotal role in facilitating essential metabolic pathways associated with oil biosynthesis. In contrast to oil content, the raw protein content of sunflower seeds, another critical nutritional attribute, exhibited a significant decline from 2022 to 2023 across most treatments (Figure 3). Specifically, the GOIM+EM treatment produced the highest protein content in 2022, whereas the K treatment was most effective in 2023, as evidenced by the significant interaction between year and fertilizer treatment. By 2024, both K+GOIM and GOIM+EM treatments were equally effective in maximizing protein concentration. These patterns underscore the importance of nitrogen application, particularly at 50% rates, in promoting amino acid biosynthesis and, consequently, protein accumulation. Importantly, treatments integrating nitrogen with organic matter facilitated a gradual and sustained nutrient release, thereby supporting continuous protein synthesis throughout the plant's developmental stages. Moreover, the use of effective microorganisms in the GOIM+EM treatment appeared to enhance nitrogen availability through mechanisms such as biological nitrogen fixation, nutrient solubilization, and stimulation of root development. Consequently, these biological processes augment plant metabolic functions critical for protein synthesis.

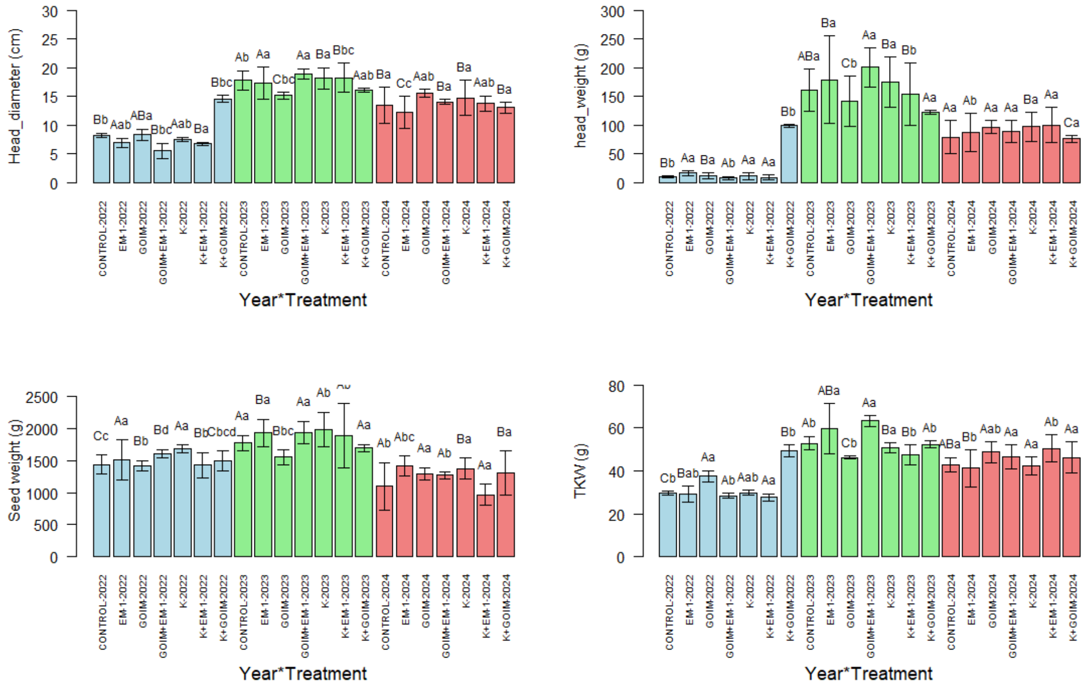

Figure 2. Effect of fertilizers Treatments on Head_diameter, Head_weight, Seed Weight and TKW across Different Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant.

Similarly, the K+GOIM treatment demonstrated that potassium supplementation significantly activated enzymatic processes linked to protein synthesis while improving nutrient translocation within plant tissues. Therefore, the synergistic combination of potassium, organic matter, and nitrogen substantially enhanced nutrient uptake efficiency, ultimately leading to improved seed protein content. Consistent with these observations, analysis of sunflower seed moisture content over the three-year period revealed significant effects of year, fertilizer treatment, and their interaction (Figure 3). Moisture levels were consistently higher across treatments in 2023, with relatively elevated values also observed in 2022 and 2024. Treatments such as K+GOIM and EM notably increased seed moisture content, suggesting that these approaches foster soil conditions conducive to enhanced nutrient availability and water retention. Enhanced seed moisture content is essential for preserving seed viability during post-harvest storage, further indicating the agronomic value of these treatments. However, a noteworthy trade-off was identified: treatments promoting higher protein and moisture levels often coincided with reductions in oil content. Specifically, the

K+GOIM treatment, while enhancing moisture and protein levels, frequently resulted in diminished oil accumulation. This phenomenon likely reflects a resource allocation shift within the plant, wherein greater nitrogen availability and higher moisture retention prioritize protein biosynthesis at the expense of lipid formation. Moreover, elevated moisture levels, by delaying seed maturation processes, may interfere with the typical accumulation of oils that occurs as moisture loss. In conclusion, the dynamic interplay among protein content, moisture levels, and oil yield illustrates a natural prioritization strategy within sunflower physiology, driven by nutrient availability and environmental conditions. Optimizing fertilizer strategies to balance these competing quality traits remains essential for maximizing the multifunctional value of sunflower crops.

4.4.Changes in oil fatty acid composition in response to fertilizer treatments and year

The chromatographic chemical analysis of sunflower oil has enabled the identification of a variety of fatty acids, ranging from C14 to C24, as illustrated in the table. According to the statistical analysis, the fatty acid composition of sunflower oil was significantly affected by both the year and fertilizer treatment, as well as the interaction between the two.

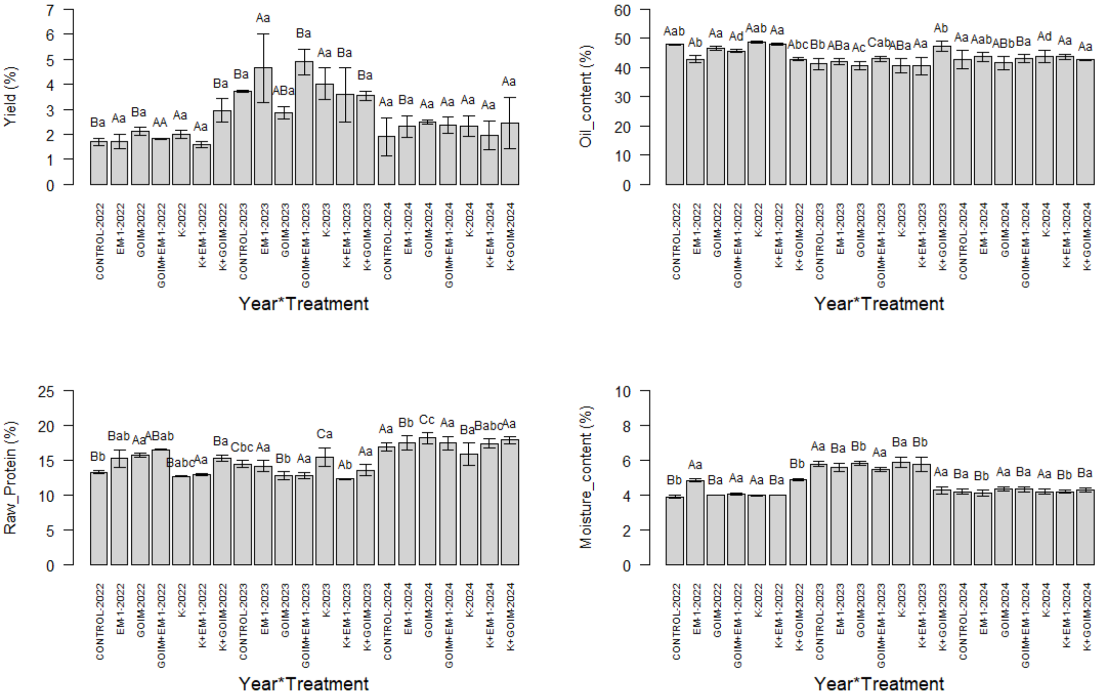

Figure 3. Effect of fertilizers Treatments on Yield, oil content, Raw protein, and Moisture Content across Different Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant.

Monounsaturated fatty acids

The study examines the impact of fertilization treatments on the quality of sunflower oil. Oleic acid, a monounsaturated fatty acid, is present in sunflower oil at an average concentration of 57-86%. The study found that oleic acid levels were significantly influenced by treatment, year, and fertilizer treatment. In 2022 and 2024, oleic acid values were higher by 70% and 84%, respectively. Significant fertilization treatments were identified, with GOIM+EM and K treatments producing the highest concentrations of oleic acid. Eicosenoic acid, another monounsaturated fatty acid, also fluctuated between 2022 and 2024. The K treatment significantly increased eicosanoid and oleic acid levels. The results indicated that various nutritional treatments exerted unique influences on the fatty acid composition of sunflower seeds, namely on eicosanoid and oleic acids, which are essential monounsaturated fatty acids critical for oil quality. The combination of organic nitrogen with inorganic nitrogen and effective microorganisms (GOIM+EM) yielded the maximum oleic acid content, hence enhancing the

MUFA levels of the oil. The presence of efficient microorganisms may improve nutrient availability and absorption, increase nitrogen-use efficiency, and stimulate root development, all of which facilitate the synthesis of oleic acid. Organic nitrogen offers a consistent nutrient release, whilst inorganic nitrogen guarantees swift nutrient accessibility, establishing an optimal setting for enhancing oil quality. The potassium (K) treatment markedly increased the levels of both eicosenoic acid and oleic acid. This is probably attributable to potassium's essential function in plant physiological activities, including enzyme activation, photosynthesis, and nutrient transport control, all of which facilitate fatty acid production in seeds. The use of potassium enhanced the quality of sunflower oil by elevating the levels of beneficial fatty acids, including oleic acid and linoleic acid.

Saturated fatty acids

The stability and shelf life of sunflower oil are influenced by the concentration of palmitic acid C16:0, a saturated fatty acid that is frequently present in the oil. It is present in sunflower oil at an average concentration of 3.69-5.33%. The palmitic acid was significantly influenced by the year. In 2023, this acid was determined to be highly significant for all treatments, with a 19% increase. Stearic acid concentration increased from 2.71% to 4.32% over the years, with 2023 being the most significant year. Oils from K+GOIM treatment showed a 47% increase, while K experienced a 37% increase. In 2022, fertilizer treatment significantly influenced stearic acid percentage, with K+GOIM, EM, GOIM, and GOIM showing the highest values. The lignoceric acid, a long-chain saturated fatty acid that is frequently found in plant oils, including sunflowers, is present at a concentration of 0.27-0.49% in sunflower oil. Lignoceric acid experienced a substantial decline from 2022 to 2023 and a significant increase from 2022 to 2024. For all treatments, the lignoceric acid increased substantially in 2024. The oil from the treatments GOIM, GOIM+EM, K, and K+EM did not exhibit any significant difference between 2022 and 2024. The oil from treatment K recorded the highest levels of lignoceric acid (0.47%), followed by GOIM (0.42%) and GOIM+EM (0.45%). In 2023 and 2024, this acid was marginally elevated for those treatments. In 2023, a substantial increase in myristic acid (C14:0), present at 0.2-0.43 % in sunflower oil was observed in all treatments with the primary increase occurring in the oil from K and K+EM by 30%. The oil from the GOIM+EM treatment remained at the same percentage of myristic acid throughout the years, about 0.03%. In 2024, the oil from K+GOIM exhibited the lowest level of myristic acid in years, about 0.02%. Margaric acid (C17:0), a saturated fatty acid, exhibited a significant effect over the year. The oil from the GOIM, K+EM, and K+GOIM treatments was influenced by the year factor. In 2023, the oil from these treatments exhibited substantially higher Margaric acid values with 23% higher than those of 2022 and 2024. The arachidic acid (C20:0) exhibited a significant year effect for all treatments, except the oil from K and K+EM treatments, which were not significant in any of the years. The arachidic acid level in the treatments EM, GOIM, and K+GOIM increased significantly over the years by 9%. The treatments K and K+EM did not exhibit any discernible difference in terms of the observed years. The arachidic acid level in 2023 (0.28%) was lower than that of 2022 (0.31%) and 2024 (0.34%). Nevertheless, the arachidic acid value was significantly higher in 2022 in the GOIM+EM treatment (0.37%). An interaction between the year and fertilizer treatment was found to be significant for this acid. The year and fertilizer treatment in 2022 resulted in higher values of arachidic acid in oil from GOIM+EM (0.37%), followed by GOIM (0.33%) and K (0.33%). These treatments generated the maximum levels of arachidonic acid in 2024, and no single treatment was significantly superior to the others. Tricosanoic acid C23:0 exhibited a significant year effect. Tricosanoic acid levels were observed to be lower in 2023 than in 2022 and 2024. For the oil from GOIM+EM, GOIM, and K, the interaction effect of year and fertilizer was significantly higher. The year had a significant effect on the Behenic acid C22:0. The oils from EM, GOIM, K+GOIM, and control treatments experienced an 11% increase in Behenic acid levels from 2022 to 2024, primarily due to the year factor.

Poly-unstaurated fatty acids

Linoleic acid C18:2, a critical polyunsaturated fatty acid that is primarily present in sunflower oil, is present in sunflower oil at a percentage of 3.62-32%. Linoleic acid was significantly influenced by the year and treatments. Additionally, there was a significant interaction effect between the year and fertilizer treatment. The linoleic acid experienced a substantial increase of 67% from 2022 to 2023 and a significant decrease of 80% in 2024. The linoleic acid percentage achieved its greatest value in 2023, about 50-60%. In comparison to the other treatments, the oils from the EM (32.1%) and K+GOIM (31.9%) treatments exhibited the maximum levels of linoleic acid in 2022. The treatment GOIM+EM yielded the lowest linoleic values, about 10.68%. α-Linolenic acid (C18:3), a polyunsaturated fatty acid that is primarily present in sunflower oil, was markedly higher in 2023 for all treatments, followed by 2022 and 2024. The interaction between the year and treatment significantly increased the concentration of α-linolenic acid in oil from K+GOIM (0.045%), followed by EM (0.043%), GOIM+EM (0.04%), and K+EM (0.04%) ($F(2.6)=3.776$, $p=0.001$). In comparison to other treatments, GOIM treatment achieved the lowest values of α-linolenic acid (0.03%) in 2022. In conclusion, the K+GOIM (potassium + organic N mixed with inorganic N) and EM (effective microorganism) treatments produced the highest levels of linoleic and alpha-linolenic acids. Meanwhile, the GOIM and K treatments specifically increased linolenic acid levels. On the other hand, the GOIM+EM-1 (organic N mixed with inorganic N + effective microorganism) and K+EM (potassium + effective microorganism) treatments enhanced alpha-linolenic acid content, although alpha-linolenic acid contributes only a small percentage to sunflower oil compared to linoleic acid. The combination of potassium, which facilitates enzyme activation, and organic matter, which improves soil fertility and microbial activity, would stimulate the metabolic pathways responsible for the synthesis of polyunsaturated fatty acids (PUFA). Potassium and organic matter enhance the plant's capacity to assimilate nutrients and stimulate enzymes associated with lipid metabolism. Potassium regulates osmotic equilibrium and enzyme activity in plants, facilitating fatty acid desaturation and elongation activities, resulting in the formation of polyunsaturated fatty acids (PUFA). The drought was more severe in 2022 than in 2023 and 2024. Linoleic acid and palmitic acid levels increased, but oleic acid levels fell, with no alterations in stearic acid levels. The greatest amounts of linoleic and alpha-linolenic acids, as well as moderate oleic acid, were found in sunflower oil when potassium was combined with organic and inorganic nitrogen (K+GOIM) and effective microorganisms (EM) treatments were used. This, in turn, led to a rise in PUFA. Linoleic acid, a principal polyunsaturated fatty acid, is essential for oil quality, but alpha-linolenic acid, despite its lesser abundance, has considerable nutritional value as an omega-3 fatty acid. The rise in linolenic acid indicates that certain fertilizer combinations may enhance the activity of enzymes involved in its manufacture, hence improving the oil's nutritional profile. GOIM+EM-1 increased oleic acid but had lower linoleic acid levels in 2022, while EM-1 increased linoleic acid but had lower oleic acid levels. The linoleic, alpha linolenic, and stearic acids were elevated by the effective microorganism, which was abundant in bacteria. The synthesis of fatty acids is further supported by activating enzymes (such as desaturases) and the enhanced nutrient absorption by microorganisms.

4.5.Effect of fertilizers treatment and year on the stability of oil

The identified fatty acids were grouped as follows: saturated fatty acids (SFA) — myristic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid, tricosanoic acid, and lignoceric acid; monounsaturated fatty acids (MUFA) — oleic acid, palmitoleic acid, and eicosenoic acid; and polyunsaturated fatty acids (PUFA) — linoleic acid and alpha-linolenic acid. These groups offer valuable insight into the oil's stability. Increased levels of MUFA, particularly oleic acid, generally improve the stability of oil. Conversely, PUFA contributes to the essential fatty acid content but may decrease stability due to its increased susceptibility to oxidation. The study analyzed the effects of year and fertilizer treatment on mono-unsaturated fatty acids (MUFA) and polyunsaturated PUFA (PUFA), and saturated fatty acids (SFA) in sunflower oil. A significant year effect, a treatment effect, and a significant interaction effect of year and fertilizer treatment were found in SFA, MUFA, PUFA, and MUFA/PUFA (Table 1). In 2024, the MUFA achieved its most significant values (87%), with 2022 and 2023 following afterward. The GOIM+EM (80%) and K (76.5%) treatments exhibited the highest MUFA values in comparison to the other treatments, as evidenced by the interaction between year and fertilizer treatment. The lowest values were recorded by EM (58.14%) and K+GOIM (58.19%). The oil from the GOIM+EM and K treatments was abundant in MUFA due to the highest levels of oleic acid, the primary monounsaturated fatty acid, which were recorded. The year 2023 was the most productive in terms of PUFA production, with 2022 and 2024 following closely behind. The post-hoc results of 2022 yielded the most significant results. The maximum PUFA values were observed in the EM and K+GOIM treatments in 2022, 32.14% and 32%, respectively. The treatment GOIM+EM exhibited the lowest PUFA values that were recorded, about 10.73%. The treatment K+GOIM and EM resulted in a high concentration of PUFAs in the oil, as evidenced by the highest levels of linoleic and alpha linolenic fatty acids. Similar results were found for PUFA ω-3 and PUFA ω-6 in K+GOIM and EM. The SFA was found to be significantly higher during the year 2023 than in 2022 and 2024. The treatment effect was more pronounced in the year 2022. The difference between treatments was significant, with EM (9.71%) and K+GOIM (9.78%) producing the highest values of SFA compared to the other treatments. However, the variation between all treatments was minimal. Treatment K registered the lowest value of SFA, about 9%. Given that the EM and K+GOIM treatments produced the highest levels of stearic acid, the primary saturated fatty acid, leading to the highest total SFA content compared to the other treatments. In 2022, the oils from GOIM+EM and

K exhibited the highest values of MUFA/PUFA, 7.55% and 5.37%, respectively, even though the treatment effect was not significant. The same result was found for the oleic/linolenic ratio. In 2024, the EM (26%), K+EM (24%), and K+GOIM (22%) treatments achieved the highest values for these ratios. The treatment GOIM, which consisted of 50% inorganic nitrogen and 50% organic nitrogen, resulted in a moderate increase in the levels of the majority of saturated fatty acids, including stearic acid, arachidic acid, and lignoceric acid, as well as linoleic and oleic acid. In comparison to other treatments, GOIM (50% organic fertilizer + 50% nitrogen) demonstrated a moderate increase in both oleic acid (monounsaturated fatty acid, MUFA) and linoleic acid (polyunsaturated fatty acid, PUFA) compared to other treatments. The K+GOIM treatment had the most significant impact on linoleic acid, resulting in the highest PUFA levels, while the GOIM+EM treatment produced the highest level of oleic acid across the study, making it the most effective combination for increasing MUFA. Although not the most effective, GOIM alone made a positive impact on the synthesis of both oleic and linoleic acid. This emphasizes the significance of GOIM in enhancing oil quality, although it may not achieve the same level of optimal performance as GOIM+EM or K+GOIM for specific fatty acid groups. Our findings indicate that the combination of GOIM with potassium results in an increase in linoleic acid, while the combination with EM results in an increase in oleic acid. A higher oleic acid content, but a lower linoleic acid content, appears to enhance the oxidative stability of oil. This suggests that the MUFA/PUFA and oleic/linoleic ratios are closely linked to stability. It is intriguing that the oils from the GOIM+EM treatment, followed by K and K+EM, exhibited the highest MUFA/PUFA and oleic/linoleic ratios, suggesting that these oils contribute to increased oil stability.

Table 1. Post hoc Results of Fertilizer Effects on Polyunsaturated Fatty Acids (PUFA), Monounsaturated Fatty Acids (MUFA), Saturated Fatty Acids (SFA), and MUFA/PUFA over the years

Treatment	Year	SFA	MUFA	PUFA	MUFA/PUFA
CONTROL	2022	9.14±0.125B*bc	71.183±0.19B*c	19.677±0.311B*bc	3.618±0.067A*b
	2023	10.92±0.284A*a	28.69±3.649C*a	60.39±3.831A*a	0.479±0.092B*a
	2024	8.93±0.284B*a	84.667±2.665A*a	6.403±2.398C*a	14.418±4.794AB*a
EM-1	2022	9.718±0.059B*ab	58.14±2.664B*d	32.143±2.726B*a	1.822±0.226A*c
	2023	10.737±0.09A*a	31.005±0.978C*a	58.14±0.89A*a	0.533±0.025B*a

	2024	8.7±0.106C*a	87.597±1.31A*a	3.707±1.34C*a	25.953±9.851AB*a
GOIM	2022	9.253±0.095B*abc	73.423±0.177B*bc	17.327±0.265B*bc	4.238±0.074A*a
	2023	11.03±0.305A*a	30.593±1.435C*a	58.37±1.256A*a	0.525±0.035B*a
	2024	9.19±0.323B*a	85.597±1.384A*a	5.207±1.677C*a	18.043±7.466AB*a
GOIM+EM-1	2022	9.24±0.377B*abc	80.037±1.749B*a	10.72±1.382B*d	7.558±1.077A*ab
	2023	10.8±0.201A*a	29.21±3.212C*a	59.99±3.412A*a	0.49±0.08B*a
	2024	8.637±0.319B*a	86.65±1.578A*a	4.71±1.333C*a	19.36±5.131A*a
K	2022	9.023±0.156B*c	76.65±0.891B*ab	14.327±1.047B*cd	5.373±0.465A*ab
	2023	11.27±0.209A*a	28.403±3.562C*a	60.327±3.548A*a	0.474±0.086B*a
	2024	8.647±0.191B*a	86.413±1.38A*a	4.937±1.534C*a	18.576±5.182A*a
K+EM-1	2022	9.267±0.304B*abc	74.347±1.099B*bc	16.387±0.897B*bc	4.549±0.325A*ab
	2023	11.177±0.265A*a	28.563±4.227C*a	60.26±3.972A*a	0.478±0.1B*a
	2024	8.907±0.652B*a	87.215±2.195A*a	4.19±1.83C*a	24.275±12.253AB*a
K+GOIM	2022	9.782±0.153B*a	58.19±1.141B*d	32.028±1.285B*a	1.82±0.107B*c
	2023	11.053±0.136A*a	29.593±1.533C*a	59.357±1.415A*a	0.499±0.038C*a
	2024	8.763±0.106C*a	87.17±1.062A*a	4.067±0.974C*a	22.251±5.134A*a

4.6.Treatment Effects on Agronomic Trait in Sunflower crops over three years using heatmap Figure

Based on the heatmap, the treatment K+GOIM showed a significant positive effect on head weight and seed weight, while EM showed a significant positive impact on leaf number and LAI. However, it had a substantial negative impact on seed weight. The GOIM treatment showed moderate positive changes in LAI and seed weight, while EM had some adverse effects. The K+GOIM and EM-1 treatments had the most significant positive effects on the growth and yield attributes of the sunflower crop. Additionally, the heatmap showed that EM-1 had the most significant positive effect on PUFA ω-3 and alpha-linolenic acid, while

K+GOIM increased alpha-linolenic acid by 19.7% and PUFA (ω-3) by 9.15%. GOIM+EM-1 treatment experienced the greatest reductions in linoleic acid, PUFA, and PUFA (ω-6).

4.7.AMMI PCA Analysis of Treatment Effects on Agronomic Traits Over Three Years

The AMMI PCA analysis of growth and yield parameters reveals that the response variable is significantly influenced by the year, fertilizers, and their interaction. The year effect has a high influence, suggesting variation across years. The interaction between fertilizers and year is highly significant, indicating that the impact of fertilizers is dependent on the specific year, suggesting inconsistent responses across years and unique environmental conditions. The study reveals that PC1 and PC2 account for 99.9% of the dataset's variance, making them the most crucial components for understanding patterns, while PC3 through PC9 are statistically insignificant. The treatments GOIM+EM-1-2023, EM-1-2023, K+EM-1-2023, and CONTROL-2023 are situated near the seed weight and SN/head vectors, indicating that they are effective in terms of seed weight and the number of seeds per head. Conversely, application fertilizers such as K+GOIM-2022, K-2022, and EM-1-2022 are more closely aligned with the head weight arrow, suggesting superior performance in terms of head weight. Consequently, the 2023 treatments appear to be more advantageous for optimizing seed-related characteristics, whereas certain 2022 treatments exhibit superior performance in terms of head weight. The 2024 treatments had a neutral or average performance in relation to both seed characteristics (seed weight, SN/head) and head weight, as they are not situated near any specific variables of relevance in the PCA plot. In comparison to other years, their proximity to the origin implies that they may represent balanced or less extreme outcomes. Regarding oil quality, AMMI PCA analysis reveals that fatty acid composition is significantly influenced by the year and fertilizer applications. The year significantly affects oil quality parameters, while the type of fertilizer used significantly influences oil composition. The interaction between fertilizer treatments and year is also significant. The first two principal components account for 87.3% of the total variance. The study found that the combination of K+GOIM and EM-1 fertilizers significantly influenced the polyunsaturated fatty acid (PUFA ω-3) and alpha-linoleic acid content of critical oil quality traits. These treatments increased beneficial oil components crucial for oil nutritional quality. However, other fertilizer-year combinations had a more balanced effect on oil quality parameters. The most promising treatments for improving oil quality in 2022 were K+GOIM and EM-1, indicating that optimal fertilizer application and favorable environmental conditions can significantly enhance oil nutritional quality. However, the efficacy of these fertilizers is limited to special conditions in the driest year, 2022, and their effectiveness diminishes in subsequent years, emphasizing the importance of considering the environmental variability that fluctuates from year to year when formulating fertilization strategies for sunflower crops. Fertilizer strategy should be preceded by annual soil fertility and microbial activity assessments. This represents a limitation of the current study, and we suggest that future research should incorporate soil microbiological analyses to better understand the interactions between microbial communities, fertilization regimes, and plant stress responses. Conducting these assessments regularly would yield valuable insights, enabling the development of evidence-based, region-specific fertilizer recommendations. Such efforts would empower sunflower producers to make more informed nutrient management decisions in response to dynamic field conditions.

5. CONCLUSION AND RECOMMENDATIONS

5.1.Conclusions

The investigation on germination determined that the optimal temperature range for sunflower seed germination is 15–30 °C, with the maximum germination rate occurring at 15 °C. The optimal temperature range for early seedling growth was 15–35 °C, with 25 °C fostering the most vigorous seedling development. The Thousand Kernel Weight (TKW) method was found to be effective in approximating the minimum optimal water requirement for germination and seedling growth under controlled conditions, as it corresponds water volume with seed size and weight. Nevertheless, these findings were obtained using Petri dishes and, as a result, cannot be directly applied to field conditions. In the latter, factors such as soil type, texture, and field capacity significantly influence water availability and retention. The germination performance of the seeds in a Petri dish was also influenced by the number of seeds, underscoring the significance of spacing in controlled experiments. The direct administration of fungicide to seeds, even as a preparatory treatment, had a detrimental effect on germination and seedling development in the fungal growth control experiment due to its phytotoxic effects. Conversely, the vigor of the seedlings was not compromised, and the fungal development during germination was effectively reduced by seed priming with sterile water or treatment with sodium hypochlorite**.**

The growth and physiological parameters of sunflower crops are considerably enhanced under rainfed conditions as a result of unique interactions between fertilizers. In 2022, the combination of potassium with both inorganic and organic fertilizers (K+GOIM) yielded significant results during a particularly severe drought period. This treatment significantly increased the number of leaves, the diameter of the stem, the chlorophyll content (SPAD), and the leaf area index (LAI). Furthermore, the use of effective microorganisms (EM-1) resulted in an even greater increase in leaf number and chlorophyll content. Additionally, the application of potassium and GOIM separately increased the stem diameter.

The K+GOIM fertilizer treatment in 2022 substantially enhanced sunflower yield parameters, while GOIM+EM exhibited a comparable effect in 2023. These results were corroborated by both the AMMI PCA analysis of yield attributes and the multivariate two-way ANOVA. The heatmap analysis further verified the substantial increase in head weight and seed weight that K+GOIM achieved. The application of potassium K either alone or in combination with EM resulted in a substantial increase in oil content in terms of quality characteristics. Moisture content was enhanced by both K+GOIM and EM treatments, while protein content increased significantly under both K+GOIM and GOIM+EM regimens. The applications of K+GOIM and EM increased the levels of polyunsaturated fatty acids (PUFAs), particularly linoleic and alpha-linolenic acids. In 2022, additional AMMI PCA analysis demonstrated that both interventions substantially increased the content of PUFA ω-3 and alpha-linolenic acid, a finding that was also corroborated by the heatmap. Conversely, the GOIM+EM treatment resulted in the highest levels of oleic acid, which in turn contributed to a higher concentration of monounsaturated fatty acids (MUFA). Nevertheless, the heatmap analysis also demonstrated that GOIM+EM had a detrimental impact on the levels of linoleic acid and PUFA ω-3, while simultaneously promoting the accumulation of MUFA. These patterns were consistently confirmed through multivariate two-way ANOVA, heatmap, and AMMI PCA analyses. Overall, the fertilizer K+GOIM (90 kg potassium + 100 kg nitrogen; 50% organic, 50% inorganic) is considered the most effective drought-resilient fertilizer strategy that enhances sunflower physiology, (+43%) yield, produced oil rich in 32% PUFA (high nutritional value omega-3 and 6) and 57%MUFA (Figure 4). Although its effectiveness was strongly influenced by environmental conditions.

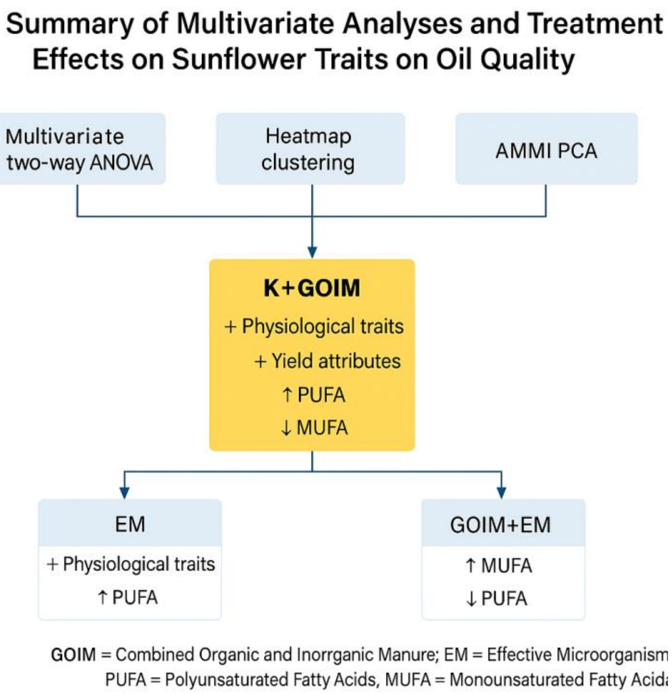

Figure 4. Fertilizers strategy effects on Fatty acid composition

1.1.Recommendations for the future

1.1.1. Recommendations based on germination experiments

- The optimal temperature for sunflower germination and seedling growth 15-30°C. In practice, target planting around the time when the soil is near 15-30°C to achieve robust seedling growth. Monitoring soil temperature with a simple thermometer can help you choose the best sowing date.
- Under controlled conditions, for studies aimed at enhancing seedling length, a water application of 8.2–11.4 ml per Petri dish is optimal. In contrast, a lower water volume of 4.4–8.4 ml should be applied when the objective is to increase the dry weight of the seedlings, thereby promoting more resilient and densely formed seedlings. It is advisable to design experiments that explore finer gradients within these ranges and measure associated physiological responses, such as osmotic potential and nutrient uptake efficiency.
- To ensure consistency in experimental setups, it is recommended to use 10–12 seeds per Petri dish, which provides an advantage in breeding programs, especially when there are seed limitations.

- Additionally, priming sunflower seeds with hypochlorite before *in vitro* germination is recommended to minimize fungal contamination. Future work should also compare the effectiveness of the priming method with other antifungal strategies to identify the optimal protocol for enhancing seed viability and uniform germination.

1.1.2. Recommendations For Fertilizer Integration Strategy

- The physiological performance, seed yield, and oil quality of sunflowers were substantially improved during drought years by the application of K+GOIM (90 kg/ha potassium and 100 kg/ha nitrogen, with nitrogen divided 50% organic and 50% inorganic). It is advisable to assess the long-term effects of this treatment across a variety of climatic conditions, including non-drought years, in order to guarantee a more comprehensive and consistent efficacy. Furthermore, future research should concentrate on the optimization of nutrient input levels, either by increasing or decreasing, to determine the optimal nutrient range for optimizing crop performance and resource efficiency in response to changing environmental conditions.
- The application of Effective Microorganisms (EM-1) led to a substantial increase in the number of leaves and chlorophyll content, suggesting that it has the potential to improve the overall vigor of crops and the growth of plants. These advantages are probably the result of improved nutrient absorption and increased soil microbial activity. To promote healthier and more productive sunflower crops, farmers are advised to incorporate EM-1 into their fertilization programs, particularly in the presence of variable or stressed field conditions. Future research should investigate the application of EM interventions in a variety of soil types, cropping systems, and environmental conditions in order to gain a comprehensive understanding of their long-term effects and broader applicability. Additionally, studies should endeavor to optimize EM-1 input levels in order to ascertain the most cost-effective and efficient application rates for sustainable crop production.
- Stem diameter, seed yield, oil content, and the concentration of monounsaturated fatty acids—particularly oleic acid and eicosenoic acid—were all significantly enhanced by potassium K application during the driest season. Based on these findings, it is advised that producers incorporate potassium at a rate of 90 kg/ha into their sunflower fertilization program, particularly in nutrient-limited or rainfed environments.
- GOIM is a key fertilizer in the integration strategy; it is advisable to combine it with potassium K to produce oil that is higher in linoleic acid, and it can be combined with EM to produce oil that is higher in oleic acid

and MUFA. To better understand the impact of various fertilizer treatments, including K+GOIM and GOIM+EM, on the composition of sunflower oil, particularly in terms of unsaturated fatty acids, we should conduct additional research. Producing sunflower oil with desirable health benefits and a higher market value can be facilitated by comprehending these mechanisms.

- The integrated fertilizer approach, such as the K+GOIM, increased the drought resilience and oil productivity of sunflowers under rainfed conditions. This approach produced oils rich in nutritional values, PUFA (32%) and MUFA (58%). For instance, applying around 90 kg/ha of potassium in combination with a balanced nitrogen mix (50% organic and 50% inorganic). Farmers could use this strategy, with certain adjustments that might be necessary depending on the production oil goals.
    - **For high-oleic sunflower oil**, which is increasingly preferred for its stability and health profile, fertilizer regimes might need to be aligned with hybrids genetically predisposed to higher oleic content, possibly requiring altered nutrient balances or reduced nitrogen rates to avoid dilution of oleic acid.
    - **For traditional linoleic oil**, maintaining a balanced supply of nitrogen and potassium, as identified in our study, can help support PUFA synthesis

2. NEW SCIENTIFIC RESULTS

i. Water application trials identified that 8.2–11.4 ml per Petri dish accelerated germination and enhanced seedling length, while a lower volume of 4.4–8.4 ml favored the development of resilient seedlings with higher dry biomass. The use of 10–12 seeds per Petri dish, combined with a priming treatment, also proved effective in limiting fungal contamination during in vitro germination.

ii. Fertilizer effectiveness varied by year, likely due to weather differences. In the drier season, K + GOIM (90 kg/ha Potassium+ 50 kg/ha of Ammonium nitrate + 50 kg/ha of dried-pelleted cattle manure) enhanced yield traits. In contrast, the wetter season favored GOIM + EM (50 kg/ha of Ammonium nitrate + 50 kg/ha of dried-pelleted cattle manure + 40 l/ha of effective microorganism), possibly due to improved microbial activity and nutrient availability under moist conditions.

iii. The integrated application of K + GOIM (90 kg/ha Potassium+ 50 kg/ha of Ammonium nitrate + 50 kg/ha of dried-pelleted cattle manure) proved to be the most effective strategy for improving sunflower growth, physiology, yield, and oil composition under rainfed conditions. Multivariate analyses, including two-way ANOVA, heatmap clustering, and AMMI PCA, confirmed K + GOIM as the optimal fertilizer combination, although its effectiveness was strongly influenced by environmental conditions.

iv. During drought conditions, both K+GOIM and EM treatments were found to significantly elevate the levels of linoleic, alpha-linolenic acid, and polyunsaturated fatty acids (PUFAs) in the sunflower oil by 40%. In contrast, the GOIM+EM treatment reduced these fatty acids by 30% and instead slightly increased oleic acid and monounsaturated fatty acids (MUFA) by 11%, suggesting that fertilizer combinations can be tailored to manipulate the fatty acid profile toward higher oleic or linoleic acid concentrations.

v. The EM treatment alone also enhanced the drought resilience of sunflower plants, as evidenced by increased leaf number (42%), higher chlorophyll content (SPAD) (24%), and elevated levels of polyunsaturated fatty acids (PUFAs) (40%). However, when EM was combined with GOIM, the treatment (GOIM+EM) shifted the fatty acid profile toward a higher oleic acid and MUFA content (11%). Similarly, K treatment alone promoted higher levels of oleic and eicosenoic acids and MUFA, but its combination with GOIM (K+GOIM) further enhanced the accumulation of linoleic and alpha-linolenic acids as well as overall PUFA levels (40%). These results show that fertilizer interactions play a key role in determining oil composition.

vi. The stability of sunflower oil quality, measured through MUFA/PUFA and oleic/linoleic acid ratios, was consistently improved when EM was combined with either GOIM (50%) or K (20%), across different growing seasons.

6. SCIENTIFIC PUBLICATIONS

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7. LIST OF THE CONDUCTED CONFERENCE PRESENTATIONS

Haj Sghaier, A.; Binti Khaled, N.; Binti Omar, S.; Varga. A.; Kende, Z. Methodological approaches of the germination of Sunflower and Oilseed Rape in vitro. Oral presented in Youth Science Forum, 8 June 2023, Keszthely, Hungary.

Khalid, N.; **H. Sghaier, A.;** Balla, I. The role of temperature on the germination activity of leguminous crops exposed to saline conditions. Oral presented in Youth Science Forum, 8 June 2023, Keszthely, Hungary.