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

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DEDICATION

I am incredibly appreciative of Almighty for guiding me throughout this PhD journey, providing me with the courage and resilience to believe in myself, and enabling me to see it through to completion. Without His limitless benefits, this achievement would not have been feasible.

The journey toward attaining this degree was arduous and demanding, but with the unwavering support and encouragement of my loved ones, I was able to surmount every challenge that came my way.

I dedicate this dissertation to the cherished memory of my beloved parents, Houda Arfa and Kamel Haj Sghaier. Though they are no longer with me, their love, guidance, and unwavering belief in my potential continue to inspire me every day. I carry their legacy in my heart, and it is their strength that has guided me through this journey. This accomplishment is as much theirs as it is mine, and I know they would be proud.

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Lastly, I dedicated this dissertation to all my relatives and friends, whose words of wisdom and encouragement helped me stay the course and complete this work.

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TABLE OF CONTENTS

List of Figures

List of Tables

List of abbreviations

AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis of Variance
AUC	Area Under the curve
DAS	days after sowing
EM	effective microorganism
FAO	Food and Agriculture Organization of the United Nations
FM	farmyard
GOIM	Plants treated with organic and inorganic nitrogen
INM	Integrated Nutrient Management
LAI	Leaf Area Index
LSD	least significant difference
MGT	Mean Germination Time
MUFA	mono-unsaturated fatty acids
PCA	Principal Component Analysis
PUFA	Poly-unsaturated fatty acids
PUFA ω-3	Poly-unsaturated fatty acids omega-3
PUFA ω-6	Poly-unsaturated fatty acids omega-6
RDF	Recommended Dose of Fertilizer
REP	replicate
Rubisco	Ribulose bisphosphate carboxylase/oxygenase
RuBP	Ribulose 1,5-bisphosphate
SD	standard deviation
SFA	saturated fatty acids
SPSS	Statistical software suite developed by IBM
T50	time to reach 50% germination
TKW	Thousand Kernel Weight
TMGR	Time at Maximum Germination Rate

1. INTRODUCTION

The sunflower (*Helianthus annuus* L.) is a globally significant oilseed crop, ranking fourth in profitability after soybean, oil palm, and canola (ADELEKE and BABALOLA, 2020; MASVODZA et al., 2015). Native to North America and introduced to Europe in the 16th century (FUTÓ, 2019; VÍGH et al., 2010), sunflower now occupies approximately 28 million hectares worldwide, contributing about 8% of global oilseed production (FAO, 2022). Its adaptability to diverse climates, including drought-prone and semi-arid regions, makes it particularly valuable in countries like Hungary, where it represents a major agricultural crop (HARSÁNYI et al., 2021).

Despite its moderate drought tolerance, sunflower production remains sensitive to water stress, particularly during germination, early growth, flowering, and seed filling stages (HUSSAIN et al., 2018; GARCÍA-LÓPEZ et al., 2014). Seed germination is a critical stage that influences crop establishment, nutrient uptake, stress tolerance, and ultimately yield (GUNATHUNGA et al., 2024; Rahman et al., 2023). Suboptimal soil moisture and temperature can cause delayed, irregular, or poor germination (KHAEIM et al., 2019; MARAGHNI et al., 2010).

In later stages, fertilization plays a key role in improving sunflower growth, yield, and oil quality. Balanced applications of nutrients particularly nitrogen and potassium, have been shown to improve stress resilience and productivity (HUSSAIN et al., 2016). Organic inputs such as farmyard manure enhance soil structure and moisture retention (HAMZA and ABD-ELHADY, 2010), and integrated nutrient management (INM), which combines organic and inorganic sources, is gaining attention as a sustainable alternative to chemical-only fertilization (CHEN, 2006; PARAMESH et al., 2023; VANLAUWE et al., 2010).

While previous studies have examined the influence of environmental factors on sunflower germination and the impact of fertilizers on yield, few have systematically optimized germination conditions under controlled environments. Even fewer have assessed long-term field responses to integrated fertilization practices in sunflowers. Existing research often focuses on either physiological or agronomic aspects in isolation, without linking early-stage seed behavior to later productivity under stress conditions. Furthermore, there is limited data on the use of combined organic and inorganic fertilizers in multi-year field experiments, particularly in semi-arid regions. This study addresses these

gaps through two independent but complementary investigations: one focused on in vitro germination optimization, and the other on integrated nutrient management in field conditions.

This study takes a two-fold approach to improving sunflower performance:

- First, it aims to optimize germination conditions (temperature, water, seed number, fungal growth) for sunflower seeds under in vitro conditions.

- Second, it evaluates the effects of organic and inorganic fertilizers on sunflower growth and oil quality under field conditions over three consecutive years, aiming to determine an appropriate integrated fertilization management strategy.

By addressing these two critical stages, germination and nutrient management separately but complementarily, this research seeks to provide a comprehensive understanding of how to enhance sunflower productivity and resilience in semi-arid and climate-challenged environments.

2. LITERATURE REVIEW

2.1. Importance of Sunflower

Sunflower (*Helianthus annuus* L.) is an important annual oilseed crop belonging to the Asteraceae family. It is native to temperate regions of North America, where it was first domesticated by indigenous peoples. After soybean, oil palm, and canola crops, sunflower is the world's fourth most profitable and cost-effective crop (ADELEKE and BABALOLA, 2020; MASVODZA et al., 2015). It is nowadays cultivated on 27.87 million hectares and produces 50.22 million metric tons, giving it an 8% share of the global oilseed market (FAO, 2022). Given its typical cultivation in temperate regions with moderate temperatures and adequate rainfall, this crop demonstrates an impressive ability to thrive in various soil compositions and climatic environments due to its sturdy and resilient nature as a rustic crop (FORLEO et al., 2018). It is considered one of the most important crops that can be grown under drought conditions**,** and has served as a "lifesaver" for the production of vegetable oil in the world, especially in arid and semi-arid climatic conditions (ARSHAD et al., 2019; SEFAOGLU et al., 2021). Additionally, sunflowers can be grown in a wide range of soil types (from sand to clay) along with a wide range of soil pH (5.7- 8). This soil adaptability, combined with the requirements of fertile soil, moderate rainfall, and viable seeds, supports successful germination and growth. The adaptation of sunflowers to different soil and climatic conditions has enhanced their cultivation as an oilseed plant throughout the world (FORLEO et al., 2018). Furthermore, it can endure approximately a 2-12 dS/m threshold of salinity (ARSHAD et al., 2019). Beyond their agronomic advantages, sunflower seeds are rich in protein and oil, which contain between 40 and 50 percent oil and between 17 and 20 percent protein. As a result, sunflowers have the potential to bridge the gap between the production and consumption of edible oil and animal feed all over the world. Niacin, vitamin E, vitamins B1 and B6, and folate are all found in high concentrations in sunflower seeds. Additionally, the seeds are an excellent source of copper, manganese, selenium, and magnesium. Phytosterols, which are found in sunflowers, contribute to a decrease in blood cholesterol levels by controlling the production of cholesterol (NGONGONI et al., 2007). The oil of sunflowers is classified as a premium oil due to the following qualities: a light color, a low concentration of linolenic acid, a moderate flavor, and a high smoke point. Sunflower oil is predominantly composed of oleic (a monounsaturated omega-9 fatty acid) and linoleic acids (a polyunsaturated omega-6 fatty acid**)** (Table 1), which collectively constitute approximately 90% of the unsaturated fatty acids and vary depending on

cultivar and processing methods (ROSA et al., 2009). Sunflower oil is known to contain additional saturated fatty acids, namely palmitic and stearic acids (ARSHAD et al., 2019).

Table 1. Fatty acid composition (%) of sunflower oil (CODEX ALIMENTARIUS COMMISSION, 2001)

fatty acid	Sunflower oil (CODEX ALIMENTARIUS COMMISSION, 2001)
Myristic acid C14:0	<0.2
palmitic acid C16:0	5.0-7.6
Palmitoleic acid C16:1	<0.3
Stearic acid C18:0	2.7-6.5
Oleic acid C18:1	14.0-39.4
Linoleic acid C18:2	48.3-74.0
Arachidic acid C20:0	0.1-0.5
α-Linolenic acid C18:3	<0.3
Eicosenoic C20:1	<0.5
Behenic acid C22:0	0.3-1.5
Erucic acid C22:1	<0.3
Lignoceric acid C24:0	<0.5
Nervonic AcidC24:1	<0.5

2.1.1. Importance of sunflowers in Hungary

Major sunflower-growing countries in the world are Russia, Argentina, several European countries, and China. Hungary is among the important producing countries of Sunflower, it ranked 8th with a production of 1,7 M tons (FAO, 2022). Hungary holds a prominent position as one of the leading producers of sunflower seeds within the continent of Europe (2%), boasting vast expanses of land exclusively dedicated to the cultivation of sunflowers (Figure 1). Throughout Europe, sunflower cultivation commenced in the 16th century (FUTÓ, 2019; VÍGH et al., 2010). In addition, the cultivation of sunflowers, which are predominantly utilized for human consumption and biodiesel, has a substantial impact on Hungary's agricultural economy. This market dynamics is inextricably linked to crude oil prices (HARSÁNYI et al., 2021; POTORI and STARK, 2015). As a result of their

pervasive recognition as one of Hungary's most significant oil-producing crops, which encompasses an extensive 691 000 hectares (FAO, 2022; HARSÁNYI et al., 2021). The production of sunflowers is extremely appropriate in Hungary due to its extensive adaptability to a variety of environmental conditions. Additionally, it can withstand the adverse effects of climate change, which is not the case with other crops. Sunflowers are suitable for cultivation in regions with well-drained soils and extended periods of scorching summers, which are frequently encountered throughout Hungary.
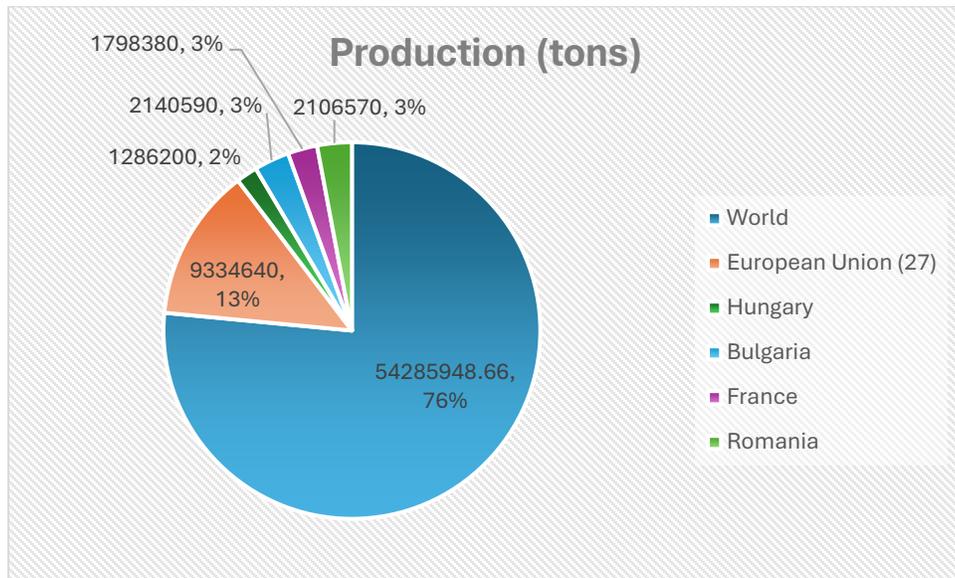
Figure 1.Sunflower production Europe (FAO, 2024)

Over the past decade, Hungary has experienced significant growth in sunflower production, primarily as a result of the improvement in the quantity and quality of sunflower hybrids, which are essential for enhancing agronomic and economic efficacy (BORBÉLYNÉ HUNYADI et al., 2008). Sunflower cultivars are essential for the improvement of yield and the development of resilience against a variety of stressors, such as environmental challenges and diseases. The evaluation of these hybrids typically concentrates on factors such as heterosis, disease resistance, and drought tolerance, in addition to the utilization of precision agriculture to optimize production.

Heterosis, or hybrid vigor, is a key principle in sunflower breeding, achieved by crossing cytoplasmic male-sterile lines with fertility restorers. This breeding strategy is fundamental for improving hybrid traits and enhancing productivity (VOLGIN and RYZHENKO, 2024). However, yield potential varies significantly across hybrids, influenced by ecological conditions and crop years (BORBÉLYNÉ HUNYADI et al., 2008). Maintaining high yields necessitates disease resistance, particularly in the face of threats such as charcoal rot, which can result in losses of up to 50% (BASHARAT, in 2024).

Breeding efforts have responded with the development of disease-resistant lines and hybrid combinations. In Hungary, the emergence of aggressive pathotypes such as *Plasmopara halstedii* pathotype 734 has posed new challenges, affecting hybrids with known resistance genes and causing moderate yield losses (NISHA et al., 2021). Continuous monitoring and the incorporation of new resistance genes are vital for managing these threats. Optimal agronomic practices are essential for maximizing hybrid performance. The recommended plant density for sunflower hybrids ranges between 45,000 and 65,000 plants per hectare; however, higher densities may increase susceptibility to diseases (SZABÓ et al., 2003). Agroecological conditions, including temperature and rainfall variations, significantly influence the performance of sunflower hybrids, affecting both disease resistance and yield. For instance, hybrids such as PR63A90 and NS-H-111 exhibit variable responses under different environmental conditions. Drought stress remains a critical concern, as it negatively impacts both yield and oil content (SIMIĆ et al., 2008). Therefore, environmental adaptability is a key criterion in hybrid selection and cultivation strategies. In addition to their agronomic advantages, some sunflower hybrids offer notable apicultural value. In southeast Hungary, a study of six hybrids identified the Coriste hybrid as the most effective in terms of nectar production and sugar concentration, making it particularly valuable for beekeeping (ZAJÁCZ et al., 2008). Moreover, certain hybrids are cultivated for specific uses, such as confectionery sunflowers in regions like Nyírség. These cultivars are prized for their favorable nutritional properties, despite being more vulnerable to pests like the sunflower moth (SZABÓ et al., 2010). This diversification reflects the growing role of sunflower hybrids in both agricultural and niche markets. Despite these advancements, weed control remains a persistent challenge in sunflower cultivation. The introduction of Clearfield® technology in 2005, especially with hybrids such as Rimisol, provided a breakthrough by enabling effective management of problematic weeds like *Ambrosia artemisiifolia* (NAGY et al., 2006). The Clearfield system represents a significant innovation in sunflower farming, utilizing imidazolinone-tolerant genotypes to facilitate post-emergence control of broadleaf weeds. This not only reduces crop competition but also enhances yield potential and supports more sustainable production practices.

2.2. The botany of the sunflower

The sunflower, an annual plant that is commonly cultivated in temperate regions and is a member of the Asteraceae family. It is characterized by the orientation of its inflorescence toward the sun. This behavior is the source of the plant's name, "Sunflower," as the head faces east in the morning and west

in the evening. However, the plant's orientation toward the sun decreases as it advances through the pollination and blossom stages (BRIGGS, 2016). The lateral roots extend longitudinally up to 60 to 150 centimeters, and the taproot system extends to depths spanning from 150 to 270 cm. The sunflower stem is characterized by its rigidity and the potential to reach heights up to 3 meters (BASHIR et al., 2015). Even though the majority of stems are unbranched, certain varieties demonstrate branching tendencies. The genetic variety of the plant and the specific conditions of its growth environment determine the dimensions, elevation, and branching patterns of the stem. Upper leaves alternate along the stem, while lower leaves are arranged oppositely (DWIVEDI, 2014). The leaves' shape and coloration vary depending on the specific species, with a range of light green to dark green colors. Once the leaves reach a length of 1 cm, the petioles of the leaves remain erect. Subsequently, the apex of the foliage progressively slopes downward as it matures. The production of leaves is abundant until the flowering stage, at which point the development of leaves decreases. The sunflower generates disc-shaped flowers at the apex of the main stem or on its branches, with flower diameters ranging from 6 to 37 cm (PUTTHA et al., 2023). The sunflower's crown is comprised of a multitude of individual florets that exhibit a unique and distinctive pattern of maturation. In the initial phases of development, these miniature flowers are arranged in a continuous spiral, with the most recently developed flowers at the center and the more mature ones at the outer borders. The florets endure a stage known as anthesis during the development process. They achieve sexual maturity within the next five to ten days, at which point they are prepared to both release and receive pollen (YOUNG-JOON and PIL JOON, 2023). The sunflower's structure corresponds to an inflorescence, which is composed of a diverse array of florets that are categorized into two distinct groups. The first category encompasses the florets that are positioned at the periphery of the flower head. The florets that are situated at the periphery of the floral head are included in the initial category. Commonly referred to as "ray flowers," these are infertile flowers that are adorned with yellow-orange sepals that emerge from the flower disk. The disk flowers, which are the second category, are perfect flowers that contain both male and female reproductive components (PUTTHA et al., 2023). The male stamens begin the process of fertilizer the female structures, which guarantees the plant's effective reproduction. It is important to observe that the frequencies of self-pollination in the majority of open-pollinated sunflower varieties are extremely low, underscoring the necessity of external pollinators to maintain genetic diversity. Sunflower seeds are composed of a kernel that is encased in a durable shell, which offers the seed protection and nutrients for its growth. Seeds that are intended for oil production are distinguished by their small size, black color, and a thin seed coat that contains a high concentration

of oil. Consequently, they are valuable for a variety of industrial and culinary applications. In terms of consumable seeds, they are significantly larger than oilseeds, and their dense hulls are readily separated from the interior, which facilitates their accessibility and consumption. This characteristic renders them optimal for breaking open to obtain the nutritious kernel, which can be employed in a variety of applications, including baking, garnishing pastries, processing into flour, or barbecuing with salt to enhance flavor (PUTTHA et al., 2023).

2.2.1. The phenological stages of sunflower

According to the research conducted by GAI et al. (2020), comprehending the ontogenetic cycle of sunflowers is essential for the modification of management practices to increase the yield of seeds and oil. As discussed in the studies by SCHNEITER and MILLER (1981) and DUANE (2007), it is essential to strategically align the crop resource demands with the prevailing environmental conditions and inputs at various growth stages. This practice is considered indispensable for achieving enhanced yield levels, and it can vary significantly depending on the specific variety and the prevailing growing environment. The BBCH scale can be used to monitor the development of sunflowers, which follows a unique pattern that defines the stages from germination to senescence. Zadoks' system provided the foundation for the BBCH scale, which was subsequently enhanced by HACK et al. (1992), organizing plant growth into 10 main phases, each with secondary stages. This system covers all phases of plant development from germination to harvest, using 2-digit codes applicable to various crops. Sunflower growth begins at the Germination and Emergence phase (00–09). During this stage, the seed undergoes a series of transformations processes, starting with the growth of a root structure that facilitates the acquisition of underground water reserves (01–03), radicle emergence (05), and followed by the subsequent emerge of shoots or above ground growth, marking the beginning of a complex developmental journey (09). The Leaf Development Stage (10–19) follows this, during which the cotyledons unfold (10), and successive true leaves begin to develop (11–19). The Side Shoot Formation Stage (20–29) is characterized by the initiation (21) and eventual maximal development (29) of side shoots as the sunflower matures. Following this, Stem Elongation (30–39) occurs, during which nodes are visible on the main stem (31) and additional elongation occurs (32–39). The floral bud becomes visible between the youngest leaves (51), and the plant subsequently transitions to the Inflorescence Emergence Stage (50–59), where it progressively reaches its maximum size and development, and ray florets become visible (59). The reproductive phase of the sunflower is initiated

by the extension of the ray florets, and the disc florets become visible (60). At stage 61, flowering begins with ray florets becoming extended. By stage 63, disc florets in the outer third of the inflorescence bloom, revealing visible stamens and stigmas. This progresses to full flowering at stage 65, where disc florets in the middle third are in bloom. At stage 67, flowering starts to decline as the inner third of disc florets bloom, and reproductive structures remain visible. Stage 69 marks the end of flowering, with most disc florets having completed their bloom and the ray florets becoming dry or falling off. The plant subsequently enters the Seed Development Stage (70–79), during which seed formation commences (71), and the seeds attain their final size and maturity (79). Finally, the Ripening Stage (80–89) is characterized by the hardening (81), physiological maturation: seeds on middle third of anthocarp dark and hard reached 60% dry matter bracts brown edge (85), and complete ripeness of the seeds, which renders them suitable for harvest (89), seeds on inner third of anthocarp dark and hard reached 85% dry matter and bracts brown. Senescence (90–99) is the concluding phase of the sunflower plant, during which it begins to discolor (91), progresses to full senescence, over-ripen, seeds over 90% dry matter (95), and ultimately desiccates completely (99). Enhanced agricultural practices, pest control, and yield optimization strategies are facilitated by the precise monitoring of sunflower phenology, which is facilitated by the BBCH scale's understanding of these growth stages.

2.3. Difficulties of sunflower production

Although sunflowers are moderately drought-tolerant, they may be susceptible to extreme drought heat stress levels when they are present, either individually or concurrently, from early flowering to achene filling. Under a highly frequent drought, even the most tolerable crop will not yield. Sunflowers encounter difficulties in managing their leaf expansion and transpiration rates during droughts as a result of inadequate soil moisture availability (GARCÍA-LÓPEZ et al., 2014). In semi-arid regions that receive minimal rainfall, the reduction in soil moisture leads to leaf wilting, which, in turn, results in a substantial decrease in yield (ABOUDRARE et al., 2006). Low yields and reduced production of sunflower hybrids can be attributed to various factors, including inadequate soil fertility, incorrect plant populations, poor weed control, diseases, insect damage, avian depredation, late sowing, and harvesting losses (ALZAMEL et al., 2022; HALL et al., 2013; IRIKA, 2015). Additionally, the unavailability of improved cultivars or hybrids, high prices of imported seed, and crop losses due to birds at maturity further contribute to these challenges (HUSSAIN et al., 2018). In numerous countries worldwide, the issue of soil fertility poses a significant challenge to the

maintenance of agricultural production and productivity. It is a frequent occurrence for cultivated lands to lack the requisite levels of vital nutrients that are essential for the production of sustainable and optimal crop yields. This inadequacy can be attributed to a variety of factors, such as soil degradation, soil acidification, a decrease in organic matter content, and compromised soil aggregate stability (ROBA, 2018). Due to the ongoing application of substantial quantities of chemical fertilizers (DAMBALE et al., 2018; MAHAPATRA et al., 2023). Furthermore, the lower productivity of sunflowers is exacerbated by the absence of high-yielding varieties and hybrids. Additionally, the cultivation of sunflowers on marginal lands with insufficient nutrients leads to poor seed setting, and the continuous application of inorganic fertilizer degrades soil health and renders it unproductive for the upcoming season (ELANKAVI, 2017a). As a consequence, cultivated areas are stagnating or decreasing, particularly in Western Europe, and the potential ecosystem services provided by the crop are insufficient to offset the lack of economic competitiveness that is predominantly due to low yields (DEBAEKE et al. 2021). Sunflowers, which are globally recognized for their economic significance and adaptability, encounter multiple challenges during cultivation. The crop's susceptibility to inadequate nutrient management in the soil is among the most pressing concerns associated with sunflower production. In addition to poor fertilization management, it is imperative to comprehend the effects of drought on sunflower growth to establish agronomic practices that will mitigate these adverse effects and guarantee sustainable production. Sunflowers are susceptible to heat stress and inadequate moisture regulation, which results in withering foliage and reduced productivity, despite their moderate drought tolerance (ABOUDRARE et al., 2006; GARCÍA-LÓPEZ et al., 2014). Sunflower production is influenced by drought-induced stress at various growth stages, with germination, anthesis, and achene filling being identified as critical phenological stages for a potential loss of up to 50% (HUSSAIN et al., 2008). The development of empty achenes may be the consequence of drought during critical stages such as anthesis (LYAKH and TOTSKY, 2014). On a global scale, these factors can result in issues with food security.

2.4. Effect of drought on sunflower growth stages

2.4.1. Germination and early stand establishment

Germination is a critical phase in the lifecycle of crops, as it establishes a basis for subsequent growth and productivity. Nevertheless, as FAROOQ et al. (2012) underscore, this stage is exceedingly

susceptible to environmental stressors, particularly inadequate rainfall at the commencement of the growing season. Crop establishment can be significantly impeded by such early-season droughts. As noted by ANGADI and ENTZ (2002) and KAYA et al. (2006), sunflower cultivation is frequently conducted in ridge and bed systems that are susceptible to limited moisture availability. Consequently, this issue is particularly pertinent. This method of cultivation may exacerbate water depletion during germination, particularly in semi-arid or rainfed regions. The physiological mechanism that supports germination is water uptake, which is contingent upon the gradient between the water potential of the adjacent sediment and the seed. ALI and ELOZEIRI (2017) confirm that effective seedling emergence is contingent upon sufficient soil moisture. Their findings are consistent with WEN (2015), who observed a proportional decrease in germination rate as soil water potential decreased. This relationship implies that even minor reductions in soil moisture can substantially impede the activation of seeds and the metabolic processes necessary for uniform emergence. It is crucial to note that the effects of water stress on germination are not limited to delayed seedling development; they can also lead to uneven and diminished crop stands, particularly in drought-stricken regions. This is further supported by EL MIDAOUI et al. (2001), who demonstrate that drought not only delays germination time but also contributes to irregular establishment, which may compromise the viability and productivity of subsequent plants. Although there is agreement regarding the susceptibility of germination to moisture deficits, the degree to which specific soil management practices can mitigate these risks in sunflower cultivation is still unexplored.

2.4.2. Plant growth and development

The growth of plants is influenced by a complex interplay of physiological and structural processes that are highly susceptible to environmental conditions, particularly abiotic stresses. Of these, drought is the most significant limiting factor after germination, as it reduces plant performance by influencing critical water-related physiological parameters, including turgor pressure, relative water content, and overall water potential (HUSSAIN et al., 2018). The osmotic imbalance that ensues initiates a series of stress responses, which ultimately impede the accumulation of biomass by reducing cell expansion and elongation. Plant growth is considerably impeded by drought-induced moisture scarcity, as affirmed by numerous studies (LISAR et al., 2012; FAROOQ et al., 2012). The accumulation of abscisic acid is a central physiological mechanism that induces stomatal closure. This response is intended to decrease transpiration, but it also limits CO_2 assimilation and, as a result, photosynthesis.

The visible symptoms of stress, such as chlorosis and wilting, are a result of the trade-off between carbon assimilation and water conservation. The plant prioritizes water retention over nutrient absorption and tissue expansion, indicating that these responses are an adaptive strategy for survival rather than productivity. This stress cascade has more extensive implications for morphological characteristics. Significant reductions in root volume and total dry matter (HUSSAIN et al., 2010), stem dry weight (HEMMATI and SOLEYMANI, 2014), and chlorophyll content (GHOBADI et al., 2013; HUSSEIN, 2011) have been associated with drought. Similarly, water scarcity results in a decrease in key growth parameters, including plant height (BURIRO et al., 2015a; ELSHEIKH et al., 2015), stem diameter (FARZAD et al., 2013), and leaf dry weight (CANAVAR et al., 2014; HEMMATI and SOLEYMANI, 2014). Net assimilation rate (HUSSAIN et al., 2010), leaf area (GHOBADI et al., 2013; GHOLAMHOSEINI et al., 2013), and canopy size are all reduced as a result of these modifications, which restrict light interception and photosynthetic efficiency, which are critical factors in yield formation. Yield losses are frequently documented during droughts. The yield of achenes is reduced as a result of reduced photosynthetic activity (GERM et al., 2005; GHOBADI et al., 2013). Additionally, biomass declines are consistently observed in various studies (LISAR et al., 2012; FAROOQ et al., 2012; FATEMI, 2014; CECHIN et al., 2015). SANTONOCETO et al. (2002) demonstrated that drought not only hinders biomass accumulation and carbon assimilation but also disrupts pollen viability and fatty acid synthesis in sunflower genotypes. Their results underscore the importance of opportune irrigation, particularly during reproductive phases, in mitigating these effects. The critical timing of water stress is frequently emphasized. ALAHDADI et al. (2011) and BURIRO et al. (2015a) emphasize that irreversible yield reductions occur as a result of inadequate irrigation during the flowering or grain filling stages, irrespective of supplementary hydration at future stages. To optimize oil yield, DEMIR et al. (2006) suggest that at least three irrigation events be implemented during heading, flowering, and kernel filling. HUSSAIN et al. (2018) quantified the effects of drought on sunflowers at various growth phases. They found that mild, moderate, and severe droughts resulted in reductions in sunflower dry matter of 45.54%, 11.70%, and 65.61%, respectively. Additionally, the total root length was substantially impacted.

2.4.3. Achene yield

Achene yield is a significant factor in the economic value and productivity of sunflower crops (RIAZ et al., 2019). Drought stress consistently and significantly reduces achene yield by affecting key yield

components, including head diameter, number of achenes per head, achene weight, and Thousand Kernel Weight (TKW) (ALAHDADI et al., 2011; FARZAD, 2013; BURIRO et al., 2015a; ELSHEIKH et al., 2015; NEZAMI et al., 2008; GHOLINEZHAD and BERNOUSI, 2009; HEMMATI and SOLEYMANI, 2014; MOBASSER et al., 2013). KHAN et al. (2000) substantiated the compound sensitivity of sunflower yield to water availability by demonstrating that an increase in drought intensity results in a substantial decline in all of these yield parameters. Water stress during vegetative stages can have a detrimental effect on growth; however, drought during reproductive stages, particularly from floral initiation to anthesis, is widely acknowledged as the most detrimental to seed production (GARCÍA-LÓPEZ et al., 2014; NAZARLI et al., 2010). Achene's formation is significantly compromised by dearth during these critical phases, which are closely associated with seed set and filling. HUSSAIN et al. (2018), for instance, reported an 83% decrease in achene yield during flowering as a result of arid stress, which was primarily caused by a decrease in TKW and achene population. The same results were reported by HUSSAIN et al. (2009), who associated yield losses during flowering with impaired fertilization and drought-induced pollen sterility. Furthermore, KAZI et al. (2002) provide additional evidence that drought restricts grain filling rates, thereby reducing the potential final yield. Collectively, these studies indicate that the magnitude of yield losses is significantly influenced by the severity and timing of drought stress, rather than its mere existence. However, late-stage stress during reproductive development directly undermines seed set, weight, and viability, while early stress affects plant architecture and resource allocation. In conclusion, sunflower yield losses are considerable due to drought stress, which reduces head diameter, achene number, and TKW, particularly during the reproductive stages. These effects underscore the necessity of guaranteeing sufficient water availability during seed filling and flowering in order to maintain the profitability of crops and the production of seeds.

2.4.4. Oil yield and quality

The fatty acid composition of sunflower seeds is substantially altered by drought stress, which impacts both the quantity and quality of the oil. A significant decrease in oil content and achene yield is observed during vegetative or reproductive stages of stress (FLAGELLA et al., 2002; HUSSAIN et al., 2008; ALI et al., 2009). The balance between oleic and linoleic acids is notably influenced by drought among the four important fatty acids, palmitic, stearic, oleic, and linoleic (MONOTTI, 2003; FLAGELLA et al., 2002). This modification is associated with the modulation of desaturase enzymes,

specifically oleoyl Δ-9 and Δ-12 desaturases, which are susceptible to environmental stimuli (LACOMBE et al., 2004). During early embryogenesis, drought stress can induce enzymatic changes that accelerate fatty acid biosynthesis and alter the composition of oil (BALDINI et al., 2002). The concentrations of oleic acid are influenced by the availability of water, which in turn affects these enzymatic pathways (BALDINI et al., 2002; ROCHE et al., 2006). Additionally, mineral imbalances during droughts may further disrupt gene expression and enzyme activity. The regulation of unsaturated fatty acid levels is also genetically controlled and cultivar-dependent, with enzyme susceptibility varying across genotypes (LACOMBE and BERVILLÉ, 2001; SCHUPPERT et al., 2006). Certain studies, including PETCU et al. (2001), reported that moderate drought stress had negligible effects on oil composition. This may be attributed to the presence of tolerant, high-oleic genotypes. In contrast, other studies have shown that drought from flowering to maturation results in an increase in oleic acid and a decrease in linoleic acid (FLAGELLA et al., 2000; ALI et al., 2009). Although cultivar-specific reactions differ, these changes may indicate an adaptive response to stress. ALI et al. (2009) observed that drought stress decreased linolenic acid and increased stearic acid in certain cultivars, while having no effect on palmitic acid. Saturated fatty acids increased, while the total unsaturated fatty acid content remained constant. GHAFFARI et al. (2023b) observed comparable trends, such as an 11% increase in palmitic acid and a 3% increase in linoleic acid, and an 11% and 6% decrease in oleic and stearic acids, respectively. These compositions are also influenced by geographic and climatic conditions. For instance, palmitic acid is more consistent across locations, while oleic acid exhibits a greater degree of variability (GHAFFARI et al., 2023a). Drought frequently results in a decrease in the total oil content, in addition to compositional alterations. Under water deficit conditions, ALI et al. (2009) observed a 10.52% decrease. The oil-to-seed yield ratio is also influenced; seed yield may be reduced more than oil concentration during moderate drought, while severe drought during flowering results in proportional declines in both (HUSSAIN et al., 2018; REDDY et al., 2003). This suggests that the most susceptible phases to water deficit is the reproductive stage, specifically flowering and seed formation, in terms of oil yield loss. Ultimately, arid stress interrupts the biosynthesis of fatty acids and the content of oil by means of both genetic and environmental mechanisms.

2.5. Impact of Drought on Physiological Traits of Sunflower Crop

Drought stress is one of the most significant environmental challenges that affects crop productivity worldwide. Plants undergo substantial physiological stress as a result of climate change and unsustainable agricultural practices, which adversely affect their growth and yield. Photosynthesis, water absorption, and nutrient absorption are among the most critical processes that are disrupted by drought. These physiological functions are indispensable for the survival and productivity of plants, as they facilitate the acquisition of essential nutrients from the soil, water regulation, and energy production. The occurrence of drought disrupts the equilibrium of these processes, resulting in reduced crop quality, lower yields, and slower growth. It is essential to comprehend the mechanisms by which drought impacts these functions in order to develop strategies that will mitigate its impact and maintain agricultural production in water-limited environments.

2.5.1. Effects of Drought on Photosynthesis

Photosynthesis is one of the most significantly impacted processes by drought stress, which initiates a series of intricate physiological and biochemical disruptions in crops. The primary cause of the decline in photosynthesis efficiency in sunflowers under arid conditions is stomatal closure, which restricts the diffusion of CO_2 and diminishes the activity of carbon-fixation enzymes like RuBisCO (GHOBADI et al., 2013; KILLI et al., 2017). POTTER and BREEN (1980) reported sunflower photosynthetic rates that varied between 25 and 32 μmol CO_2 m^{-2} s^{-1}. However, these values are unlikely to be maintained during drought conditions due to the physiological constraints imposed by reduced stomatal conductance. Despite the fact that sunflowers have bilateral stomata that theoretically facilitate CO_2 diffusion, their efficacy is significantly diminished during droughts when gas exchange is reduced to conserve water (GHOBADI et al., 2013). The effect of drought on photosynthesis is not consistent; rather, it is contingent upon environmental intensities and genotypes. GHOBADI et al. (2013) emphasized that drought stress diminishes the efficiency of photosystem II, chlorophyll content, and stomatal conductance, thereby impairing photosynthetic machinery. Critically, these responses indicate a more extensive perturbation of both the carbon assimilation and photochemical energy conversion pathways. The reduced carboxylation efficiency and lower rates of RuBP regeneration that result from the suppression of RuBisCO activity under stress conditions are particularly detrimental, as they ultimately impede the Calvin cycle. However, drought-tolerant

genotypes frequently demonstrate elevated RuBP levels during extended stress, which may indicate an adaptive mechanism for maintaining carboxylation capacity in the face of enzyme inhibition (HUSSAIN et al., 2018). This genotype-specific biochemical resilience emphasizes the significance of genetic background in the development of sunflower responses to arid conditions. This variability also underscores the necessity for breeding programs to prioritize physiological markers, such as sustained RuBP production or increased photochemical efficiency, as indicators of drought tolerance. Moreover, drought restricts photosynthesis by restricting the assimilation of CO_2; however, this effect may be partially mitigated by employing suitable fertilization strategies. The provision of essential nutrients can indirectly support increased carbon fixation under duress by promoting leaf expansion, chlorophyll synthesis, and enzymatic function. In summary, the reduction in sunflower productivity is both a cause and a consequence of the decline in photosynthetic efficacy during drought. The multifactorial character of drought tolerance in sunflower cultivation is underscored by the fact that the extent of this impact is influenced by genotype, physiological plasticity, and management practices.

2.5.2. Effects of drought on water relations

The taproot system of sunflowers is highly developed, which greatly improves its capacity to assimilate water and essential nutrients from deeper soil layers, thereby enabling it to efficiently utilize soil water resources (HUSSAIN et al., 2018). The plant's growth, development, and productivity are substantially enhanced by this adaptation. Nevertheless, sunflower is still susceptible to the adverse impacts of drought stress, which can significantly impede its physiological and developmental processes, despite these biological advantages (HUSSAIN et al., 2018). Ultimately, drought impairs cell division and decreases leaf relative water content by reducing water transport through the xylem, thereby limiting nutrient translocation via the phloem and lowering the plant's water potential, including turgor and osmotic potential (POORMOHAMMAD KIANI et al., 2006). However, drought-tolerant sunflower genotypes are capable of sustaining water and nutrient transport and maintaining a higher water potential, primarily as a result of the more efficient water absorption supporting their root systems (HUSSAIN et al., 2018).

2.5.3. Effects of drought on Nutrient uptake

Plant development is driven in concert by photosynthesis, water, and nutrients. While water helps to move nutrients and maintains cellular structure, photosynthesis turns light into chemical energy. Especially nitrogen, phosphorus, and potassium, essential nutrients sustain important physiological functions. Plants show several physiological disturbances under drought stress, including lower transpiration, compromised membrane integrity, and slowed nutrient absorption and movement (GUNES et al., 2008). These disturbances damage production and development. A basic building block of amino acids and nucleic acids, nitrogen loses availability during a drought because of slowed nitrogen mineralization in the soil (HUSSAIN et al., 2018). Further impairing nitrogen movement from roots to shoots is reduced transpiration (DU et al., 2010). As shown by HE and DIJKSTRA (2014), YUE et al. (2018), and MARIOTTE et al. (2020), phosphorus absorption is similarly limited during water stress, mostly owing to reduced soil phosphorus mobility. Furthermore, severely impacted is potassium absorption; drought-induced decreases in epidermal cell turgor pressure restrict stomatal closure, which depends on enough potassium accumulation to sustain pressure for appropriate functioning (RAHBARIAN et al., 2011; HABIBI, 2012). Furthermore affecting the absorption of other vital macro- and micronutrients, including magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and sodium (Na) are drought stress and trace elements like sulfur (S), molybdenum (Mo), and silicon (Si, among others (HUSSAIN et al., 2018). Reduced CO_2 absorption for photosynthesis results from stomatal closure brought on by water shortage, minimizing water loss, therefore affecting plant production and carbon assimilation. Concurrent reduced root function reduces water and nutrient uptake, therefore aggravating the stress load. These combined physiological impacts highlight the requirement for efficient drought-reducing techniques to maintain plant development and output under low water availability (HUSSAIN et al., 2018).

2.6.   Essential Nutrients for Sunflower Growth and Development

Nutrient management becomes as critical as water availability in maintaining plant life, development, and production under drought stress conditions. A balanced and enough nutrient supply increases plant resilience helps sustain production in water-limited conditions, and improves water use efficiency, much as water is vital for physiological activities. Fundamental functions in plant physiology,

including protein synthesis, energy transmission, and photosynthesis, are played by essential macronutrients like nitrogen, phosphorus, and potassium. Likewise, while in lesser amounts, micronutrients are very essential for metabolic control, hormone production, and enzyme activity. Important components of these processes include iron, zinc, and manganese. Macro- or micronutrient deficits can slow down growth, impair vigor, lower yield, and compromise crop quality. Therefore, it is essential to apply balanced nutrition management techniques, guaranteeing sufficient availability of both macro- and micronutrients in order to sustain production. Usually, their origin, organic or inorganic, helps to classify fertilizers. Derived from biological components, organic fertilizers deliver nutrients gradually through natural breakdown, therefore encouraging long-term, consistent nutrient availability. On the other hand, inorganic fertilizers, synthesized or derived from non-living mineral sources, offer fast and targeted nutrient replacement, therefore addressing deficits and promoting instantaneous plant response. Both kinds offer different benefits, and their combination can maximize nutrient usage efficiency, especially under stress like drought.

2.7.   Inorganic Fertilizers in Crop Production

Decline in soil organic matter has been extensively documented and usually ascribed to overgrazing and the removal of agricultural residue, therefore depleting vital elements such as nitrogen, phosphorus, and potassium. Chemical fertilizers are usually required to quickly restore fertility in circumstances of extreme soil degradation as they offer nutrients in easily soluble forms that are instantly available to plants (ROBA, 2018). Through better plant growth and biomass return (SCHOLL and NIEUWENHUIS, 2004), inorganic fertilizers have also been demonstrated to indirectly boost organic matter and enhance root residues. Modern agriculture is depending more and more on chemical fertilizers in order to boost output (NATSHEH and MOUSA, 2014). Their great nutritional value means that just little inorganic fertilizer is required to noticeably increase productivity. Quick absorption made possible by their water-soluble character stimulates quick plant development as compared to organic fertilizers. Applied properly, inorganic fertilizers can help to increase root mass and crop residue, thereby supporting organic matter buildup in the soil (CHEN, 2006; HAN et al., 2016). As SHARMA (2017) demonstrates, fertilizers are necessary for raising soil fertility and avoiding nutrient constraints that lower crop yields. All of which compromise long-term soil health and agricultural sustainability, the excessive use of chemical fertilizers is linked with a range of environmental concerns, including soil acidification, nutrient leaching, groundwater

pollution, decreased trace element availability, and greenhouse gas emissions (ADEDIRAN et al., 2005; CHEN, 2006; HAN et al., 2016). Over-application also disturbs soil microbial populations and damages helpful species engaged in nutrient cycling and breakdown. These disturbances can affect plant-root interactions, including mycorrhizal associations (ABEDI et al., 2010; CHEN, 2006; HAN et al., 2016). Nitrogen fixation is another one. The overuse of fertilizers presents potential hazards to human health and the adjacent environment, in addition to these ecological risks (ROBA, 2018). Therefore, sustainable agriculture depends on the appropriate and balanced use of fertilizers to protect ecosystem health.

2.7.1. Macronutrients

The yield of sunflower achenes, as well as the amount of kernel oil and fatty acids within them, is significantly influenced by nutrient management. This management strategy has a substantial impact on the overall productivity and quality of sunflower crops, thereby emphasizing its significance in agricultural practices.

Nitrogen

Nitrogen fertilization significantly enhances the growth, development, and productivity of sunflower plants. Numerous studies have shown that even relatively low nitrogen levels can support satisfactory oil yields. For example, DA ROCHA et al. (2020) found that sunflower cultivation could achieve high oil yields with just 30–50 kg/ha of nitrogen. Similarly, FAISUL UR et al. (2013) and RASOOL et al. (2013) reported that nitrogen rates ranging from 40 to 120 kg/ha improved plant growth and yield components, with higher rates producing superior results. These findings suggest that under certain environmental and soil conditions, minimal nitrogen inputs may be both economically and agronomically feasible. According to SOUZA et al. (2020), sunflower cultivation can remain economically viable even with increased nitrogen concentrations; however, they observed that optimal nitrogen requirements vary among varieties, typically ranging from 80 to 120 kg/ha. Despite the benefits of low nitrogen levels, many studies recommend higher applications to optimize crop productivity, seed yield, and vegetative growth. KANDIL et al. (2017) reported that 168 kg/ha significantly increased plant height, stem thickness, number of leaves, leaf area, head diameter, and thousand-kernel weight (TKW), resulting in yield increases of 12.0% and 11.6% over two years. ABDUL et al. (2019) found a crown diameter increase of 8.45 cm at 150 kg/ha compared to the

control, while SOLEYMANI (2017) recommended 100–150 kg/ha. TRAVLOS et al. (2019) observed that sunflower growth responded positively to nitrogen levels of 150–300 kg/ha. SINCIK et al. (2013) identified 145–150 kg/ha as the economically optimal rate under non-irrigated conditions, increasing to 177–190 kg/ha with irrigation. Nitrogen is essential for structural and metabolic functions in plant cells. It forms part of key biomolecules such as enzymes, nucleic acids, proteins, and amino acids (ISHAQ et al., 2014; KOUTROUBAS et al., 2020; NASIM and BANO, 2012). It supports biomass formation, carbohydrate assimilation, protein synthesis, and early vegetative growth (RASOOL et al., 2013). Sunflowers mainly assimilate nitrogen as ammonium (NH_4^+) and nitrate (NO_3^-) (OZTURK et al., 2017), which stimulates vegetative growth and seed development (NASIM et al., 2017; SCHULTZ et al., 2018). Nitrogen supplementation can increase yield and oil productivity by up to 40% (YASSEN et al., 2011; ZUBILLAGA et al., 2002), and it also improves quality attributes and growth indices (SALAMA ABDALLA et al., 2013). However, nitrogen application requires careful management. Most soils are nitrogen-deficient, necessitating supplementation for optimal yield (GOVINDASAMY et al., 2023; ZULU et al., 2023). Yet excessive nitrogen can delay budding and reduce achene yield and oil concentration (MUHAMMAD IRFAN et al., 2018; OYINLOLA et al., 2010). The form of nitrogen also matters, OZTURK et al. (2017) found that urea outperformed ammonium sulfate and ammonium nitrate for oil and achene yield. MUNIR et al. (2007) noted that inorganic nitrogen improves biomass and nutrient uptake by increasing the net assimilation rate, crop growth rate, and leaf area index. These studies collectively highlight that effective nitrogen levels for sunflowers range from 30 to over 190 kg/ha, depending on target yield, soil fertility, irrigation, and variety. While higher rates may maximize yield, they also pose risks to oil quality, plant development, and environmental sustainability. Therefore, an ecologically responsible, economically viable, and site-specific nitrogen management strategy is essential for sustainable sunflower cultivation

Phosphorus

Phosphorus is derived from phosphate rock deposits that are naturally occurring and are fundamentally a form of mined materials. Upon the treatment of phosphate concentrate with sulfuric acid (H_2SO_4), a transmutation takes place, resulting in the production of either single superphosphate or phosphoric acid. Monoammonium phosphate or diammonium phosphate is produced by combining the resultant acid with ammonia. Additionally, the concentration of phosphoric acid or the further intensification of phosphate concentration can be used to produce the fertilizer known as triple superphosphate (GREEN, 2024). As a result, the various types of phosphorus fertilizers that are commonly utilized

include single superphosphate, triple superphosphate, mono-ammonium phosphate, di-ammonium phosphate, and ammonium polyphosphate liquid (JOHNSTON et al., 2014). Phosphorus is an important fertilizer to the growth and development of plants, occupying the second position after nitrogen. The plant's ability to regulate a variety of physiological reactions and improve its resistance to abiotic stresses, including high concentrations of carbon dioxide, heat, salinity, dehydration, waterlogging, and heavy metal toxicity, is contingent upon the presence of phosphorus (HUANG et al., 2020; JIN et al., 2015; LAMBERS, 2022; MALHOTRA et al., 2018). Supplementing plants with soluble phosphate form phosphorus pentoxide (P_2O_5) during all phases of their development is a practice that is widely advised (BINDRABAN et al., 2020). In the study conducted by KALAIYARASAN et al. (2017), it was determined that the application of P_2O_5 at a rate of 100 kg/ha resulted in the highest levels of seed yield, stalk yield, oil content, and oil yield of sunflower. Similarly, a study by RAVISHANKAR and MALLIGAWAD (2017) found that the application of 126 kg/ha N and 90 kg/ha P_2O_5, with a N/P fertilizer ratio of 1.40, led to notably higher head diameter, seed yield, seed oil content, and oil yield compared to the control group. This suggests that the specific combination of nitrogen and phosphorus fertilizers, along with the appropriate N/P ratio, plays a key role in enhancing the growth and productivity of the crop under investigation. Moreover, the use of nitrogen in isolation, particularly at high concentrations, might not yield substantial improvements in growth and productivity (ZANGANI et al., 2021). The plant requires an optimal level of phosphorus at the right form and at the right time, at the initial stages to ensure the sustained and efficient production of crops, as highlighted in the research conducted by GRANT et al. (2005). Nevertheless, diminished agricultural outputs can be the result of insufficient phosphate levels in the soil, which can have adverse effects on seed production, vegetative phase quality attributes, and root development (ABOBATTA and ABD ALLA, 2023; LOPEZ et al., 2023).

Potassium

Potassium is indispensable in the physiological processes of plants. It plays a critical function in photosynthesis and transpiration by regulating stomatal movement, facilitating CO_2 assimilation, and regulating water uptake through osmotic pull. Furthermore, it enhances the efficiency of nutrient transport and detoxification mechanisms within plant tissues, as well as modulates enzyme activity, protein synthesis, and pH balance in cells (MUHAMMAD IRFAN et al., 2018; ZAHOOR et al., 2017b; LU et al., 2017). These physiological advantages result in direct enhancements in seed quality and biomass accumulation, which serve as the structural and metabolic foundation of plant

development (LI et al., 2018; ZAHOOR et al., 2017a). It is notable that potassium considerably improves a plant's capacity to endure abiotic stresses, such as drought, heat, and salinity, by regulating the generation of reactive oxygen species (ROS), maintaining cell turgor pressure, and fostering osmolyte accumulation. These adaptive traits are especially pertinent in the cultivation of sunflowers, as water scarcity is a constraining factor. Enhanced potassium nutrition improves photosynthetic efficiency and stomatal conductance, and it works in conjunction with nitrogen to optimize stress tolerance (BUKHSH et al., 2012; LU et al., 2017; MUHAMMAD et al., 2018; REDDY, 2004; SOLEIMANZADEH et al., 2010; EGILLA et al., 2005; FARID et al., 2018; JÁKLI et al., 2017). Potassium has been demonstrated to improve the quality and yield of sunflowers, particularly when used at optimal levels, from an agronomic perspective. Potassium fertilization is linked to enhancements in biological yield, head diameter, and seed oil content, as per CHAJJRO et al. (2013); DIPAK et al. (2018); and MOHAMMADI et al. (2013). Nevertheless, deficiencies can reduce the efficacy of water use, rendering plants more susceptible to drought (AHMAD et al., 2018; GUJAR et al., 2018; ZAHOOR et al., 2017a). It is intriguing that the assimilation of potassium in sunflowers can surpass or equal that of nitrogen or phosphorus, as demonstrated by LI et al. (2018) and TABASSUM (2021). This underscores the critical role of potassium in nutrient dynamics. The response to potassium is highly cultivar-specific and dose-dependent, despite these benefits. Four sunflower varieties were evaluated in a study conducted by DOS SANTOS et al. (2023) on K_2O levels that varied from 0 to 120 kg/ha. The optimal range for nutrient efficacy was identified as 75 to 91 kg/ha, indicating that productivity may be compromised by over- or under-application. DAR et al. (2021) discovered that a K dosage of 150 kg/ha under optimal conditions maximized oil content, achene weight, and macronutrient uptake in another comprehensive trial. The significance of site-specific nutrient management, which is determined by environmental conditions and varietal responses, is underscored by these findings. Additionally, the effects of potassium are enhanced when it is administered in conjunction with other nutrients, particularly nitrogen and micronutrients. For example, HUSSAIN et al. (2016) reported that the co-application of K and N resulted in improved antioxidant activity and osmotic adjustment, which in turn increased drought resistance and yield. In the same vein, ZAFAR (2024) demonstrated that the combined application of 60 kg/ha K and 15 kg/ha Fe substantially enhanced morphological and yield parameters, such as plant height, head diameter, and 1000-kernel weight. In conclusion, potassium is not only a nutrient that provides support; it is essential for the productivity of sunflowers, especially in environments that are susceptible to stress. However, the complete potential of this substance necessitates a precise equilibrium: a sufficient supply, the correct

form, punctual application, and synchronization with other nutrients. The trinity of essential macronutrients in agriculture is, in general, composed of nitrogen, phosphorus, and potassium, which are derived from natural gas, phosphate rock, and potash rock, respectively. The necessity of environmentally responsible, efficient fertilizer strategies to guarantee sustainable crop production in sunflowers and beyond is emphasized by their intricate physiological functions and production processes.

2.7.2. Micronutrients

Micronutrients are essential for the acceleration of plant maturity, enzyme activation, and cell division, despite their requirement in trace quantities. In soils with low fertility or protracted intensive cultivation, their deficiency poses a critical constraint on both crop yield and nutritional quality, despite their minimal quantitative requirement (JAGASIA et al., 2023). The micronutrient sufficiency of the soil is frequently indicated by the visible heath of plants. Targeted micronutrient supplementation has been demonstrated to substantially improve the physiological resilience and productivity of sunflower plants in drought stress conditions (BABAEIAN and TAVASSOLI, 2011; SHEHZAD et al., 2016; ZAFAR et al., 2014). These benefits include enhanced antioxidant defense mechanisms, delayed leaf senescence, increased achene weight, and improved oil yield. In particular, BABAEIAN and TAVASSOLI (2011) found that the biochemical basis of drought tolerance was underscored by a 48–89% increase in critical antioxidant enzymes—superoxide dismutase, catalase, and glutathione peroxidase—as a consequence of a combined application of iron, zinc, copper, and manganese. This evidence emphasizes that micronutrients enhance crop performance in a disproportionately large way, even in minimal quantities, particularly under abiotic stress conditions like drought.

2.8. Organic fertilizers in Crop Production

Organic fertilizers, which are derived from botanical or zoological sources such as livestock manure, green manure, crop residues, and household waste, serve a dual purpose in agriculture. They not only replenish essential nutrients but also improve soil structure, microbial activity, and ecosystem health (NATSHEH and MOUSA, 2014). Their mechanism of action entails the incremental decomposition of organic matter by soil microorganisms, which leads to the slow and continuous release of nutrients

in a form that is accessible to plants (AMUJOYEGBE et al., 2007). The complexity and connection of soil-plant-microbe systems, which are central to the sciences of organic farming, a rapidly expanding approach that aims to balance the interactions between soil, flora, fauna, and microbial life for long-term sustainability are reflected in this biological mechanism (BEROVA et al., 2010). Numerous studies have emphasized the ability of organic fertilizers to enhance crop quality and productivity to a level that is comparable to that of synthetic inputs. For example, BULLUCK et al. (2002) illustrated their beneficial influence on crop productivity, indicating that organic inputs may serve as a substitute or complement to mineral fertilizers when utilized appropriately. Another frequently cited advantage of organic fertilizers is their environmental safety. In contrast to certain chemical fertilizers, which may contribute to soil acidification, leaching, or microbial disruption, organic fertilizers improve soil fertility by increasing microbial diversity, improving porosity, and raising organic matter levels—all without introducing a risk of root burn or harming beneficial organisms (HAMZA and ABD-ELHADY, 2010). In this context, their ability to enhance water retention, prevent erosion, mitigate soil pH, and promote disease resistance has been extensively demonstrated (AKHTAR et al., 2009; HAMZA and ABD-ELHADY, 2010; HAN et al., 2016; LAL, 2006; OLANIYI and AJIBOLA, 2008; NATSHEH and MOUSA, 2014). Collectively, these advantages foster a soil that is more biologically active and resilient, which in turn facilitates the development of robust plants and the provision of enhanced ecosystem services. For example, SARKAR et al. (2003) underscore the fact that organic fertilizers facilitate long-term fertility by means of incremental nutrient release, a characteristic that guarantees a consistent nutrient supply throughout the cropping cycle. Nevertheless, this slow-release property is a double-edged sword. It also presents a temporal mismatch with crop nutrient demand, particularly in early growth stages, despite the fact that it promotes nutrient availability over time. This delay in nutrient availability may result in initial deficiencies during critical developmental phases (CHEN, 2006; MORRIS et al., 2007). The rate of decomposition is highly dependent on environmental variables, such as soil moisture and temperature. This phenomenon, nutrient immobilization, is particularly problematic for fast-growing or nutrient-demanding crops, as it occurs prior to the effective onset of mineralization. Furthermore, the practical constraints of organic fertilizer use, particularly in smallholder systems, are substantial. In areas where organic matter resources are scarce, their lower level of nutrients necessitates increased application volumes, management challenges, transportation costs, and labor demands (MORRIS et al., 2007). Furthermore, organic fertilizers may contain pathogenic microorganisms if they are not correctly composted, which can pose health dangers to both plants and

consumers. This is particularly pertinent for inputs that are derived from contaminated organic matter or animal waste, as demonstrated by CHEN (2006). In conclusion, organic fertilizers provide evident ecological and agronomic benefits; however, their efficacy is contingent upon the specific circumstances. In order to mitigate risks and optimize performance, their integration into fertilization regimens must take into account climatic conditions, crop growth stages, organic matter availability, and appropriate decomposition techniques. The most practical approach to sustainable and productive agriculture may involve a balanced approach that potentially incorporates both organic and inorganic inputs.

2.8.1. Biochar and biochar fertilizers

The integration of biochar with conventional nutrient sources in biochar-based fertilizers provides a multifaceted approach to enhancing soil fertility and fostering sustainable agriculture. These fertilizers address one of the primary inefficiencies of traditional fertilizers by significantly enhancing nutrient retention and minimizing nutrient leaching by utilizing the high porosity and adsorptive capacity of biochar. Additionally, the soil's cation exchange capacity and nutrient availability are enhanced by biochar's capacity to stimulate microbial activity, which in turn enhances plant uptake. Additionally, these formulations promote beneficial biological processes that promote long-term soil health, in addition to enhancing the physical structure and chemical reactivity of the soil. In a critical manner, biochar-based fertilizers can reduce the risk of contamination in the food chain by restricting the bioavailability and plant uptake of harmful substances, including pharmaceuticals, engineered nanomaterials, pesticides, heavy metals, and polycyclic aromatic hydrocarbons (HUSSAIN et al., 2018). Consequently, they are a strategic innovation in nutrient management that connects environmental safety with productivity.

2.8.2. Biofertilizers

Bio-fertilizers, which are also referred to as microbial inoculants, are natural substances that are made up of a variety of beneficial microbes and fungi. By facilitating nutrient availability and uptake from limited soil nutrient pools, their inclusion positively influences plant growth and enhances soil biodiversity (KUMAR et al., 2017). In addition, biofertilizers provide an economically viable and environmentally sustainable alternative to synthetic inputs by enhancing the quality and quantity of

crops (DURAID et al., 2019; GOUDA et al., 2018; MĄCIK et al., 2020). Nevertheless, their efficacy is contingent upon the metabolic activity of the microorganisms and their type during and after field application (GOUDA et al., 2018; MĄCIK et al., 2020). According to DURAID et al. (2019), the highest levels of nitrogen, protein content, and yield parameters were observed in biofertilizer treatments that contained a high concentration of Bactrian mixed. HIGA (1991) developed Effective Microorganisms (EM), which is one of the most extensively researched and commercially utilized biofertilizers. In healthy ecosystems, a synergistic combination of beneficial microorganisms, such as photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes, and fermenting fungi, naturally coexist. This is known as EM. The introduction of EM has demonstrated the potential to improve crop quality and yield, enhance populations of beneficial organisms, and reduce soil-borne diseases, particularly in water-stressed conditions (HIGA, 1991; HUSSEIN, 2024). However, the efficacy of EM is contingent upon the type of soil, crop species, and application method. For example, the application of EM significantly increased the yield of sweet potatoes during dry seasons (YOUNAS et al., 2022). Additionally, it was discovered that EM increased chlorophyll content and photosynthetic efficiency, which in turn facilitated nutrient assimilation and plant growth (ABU-QAOUD et al., 2021; EL-KHATEE et al., 2018; IRITI et al., 2019; JIANG et al., 2024; TALAAT, 2019; YOUSSEF et al., 2021). Furthermore, EM compost has been linked to increased biological activity, such as increased nematode populations and improved wheat yields, in comparison to traditional compost (HU and QI, 2013).

2.8.3. Compost

Compost, which is produced through the decomposition of plant and animal residues, is a valuable organic amendment that has the potential to enhance the health of soil in numerous ways. Composts generated from manure are frequently integrated into agricultural soils to optimize their chemical composition. This enhancement is the result of the high organic matter content in compost, which serves as a nutrient reservoir, facilitates nutrient cycling, and enhances the cation exchange capacity and soil pH. In addition to its chemical advantages, compost enhances soil physical characteristics by reducing bulk density, increasing porosity, improving aggregation, and improving root penetration, water infiltration, and moisture retention. Moreover, compost is essential for the maintenance of soil biological health by promoting the activity of soil macrofauna, which are essential indicators and drivers of soil quality. The application of compost, which is a rich source of essential macro- and

micronutrients, particularly nitrogen (N), phosphorus (P), and potassium (K), ultimately contributes to improved plant growth and long-term soil fertility (GOLDAN et al., 2023).

2.8.4. Green waste or biowaste

Biowaste, which is frequently referred to as "green waste," is derived from a variety of sources, including food debris, household organic waste, and crop residues. Nevertheless, it specifically excludes materials such as forestry and agricultural residues, animal manure, sewage sludge, and other biodegradable wastes, such as natural textiles, paper, or treated wood. The agronomic value of green waste is restricted by its typically low nutrient density, despite the fact that its application can improve soil physical structure by increasing porosity and organic matter content. Additionally, the utilization of untreated biowaste may result in potential phytosanitary hazards, as it may function as a vector for plant pathogens (DINCĂ et al., 2022). Consequently, its application necessitates caution and frequently necessitates additional processing or composting to guarantee its safety and efficacy in agricultural environments.

2.8.5. Manure

Manure, which is primarily derived from livestock, including cattle, pigs, and poultry, has been utilized for a long time to improve soil fertility. This is due to its ability to improve the physical, chemical, and biological properties of soil (INDRARATNE et al., 2009). Its agronomic advantages are derived from its composition, which comprises bicarbonates, organic anions, and basic cations. These compounds have the capacity to buffer soil acidity, improve pH balance, and increase nutrient availability and water retention. Additionally, manure enhances the cation-exchange capacity of the soil by increasing the content of carbon and organic matter, which in turn facilitates nutrient cycling and nitrogen mineralization. The sustained release of nutrients is facilitated by the gradual decomposition of organic material in manure, which contributes to the long-term restoration and fertility of the soil. Manure also enhances soil biodiversity by establishing conditions that encourage the growth of beneficial microbial communities, including *Actinobacteria*, and by suppressing pathogens and pests through the release of allochemicals and microbial antagonism (GOLDAN et al., 2023). Nevertheless, the application of this technology is complicated by a number of concerns, despite its numerous benefits. Manure may contain potentially hazardous elements, such as

pharmaceutical residues and heavy metals such as copper and zinc, which are frequently present as a result of their inclusion in animal feed. These elements may contribute to the dissemination of antibiotic resistance genes. Additionally, excessive application, particularly in regions with intensive livestock production, can result in the leaching of nitrate and phosphorus, which can lead to water contamination and eutrophication risks (LOYON, 2018; RAYNE and AULA, 2020). Careful management and regulation of manure use are required to ensure both environmental protection and agronomic benefit in light of these risks.

2.8.6. Vermicompost

Vermicompost, the biologically active excreta of epigeic earthworms, is becoming more widely recognized as an effective organic amendment. This is due to its ability to enhance the microbial ecology, structure, and fertility of soil. According to ADHIKARY (2012), vermicompost is significantly concentrated in plant-available nutrients and microbial populations than typical topsoil or potting soil mixes. It is reported to contain 1.5 times more calcium, 5 times more nitrogen, and 7 times more potash, with a nutrient longevity that exceeds that of standard mixtures by up to six times. The worm's digestive system converts phosphorus into bioavailable forms, thereby improving the uptake of plant nutrients, as a result of the passage of organic material. In addition to its nutritional profile, vermicompost enhances the structural properties and water retention of soil, while also promoting long-term soil health by introducing beneficial microbial communities. Nutrient cycling, disease suppression, and overall plant vitality are all significantly influenced by these microbes. Nevertheless, the efficacy of vermicompost is context-dependent, and it is influenced by factors such as the type of feedstock, the species of earthworms, and the environmental conditions that exist during the composting process. Organic waste materials, such as sewage sludge, animal manure, agricultural residues, and digestate, are processed through a synergistic interaction between earthworms and microorganisms in the bio-oxidative degradation process of vermicomposting. This results in a stabilized compost that is abundant in nutrients, enzymes, vitamins, growth regulators, and microbial antagonists, which effectively suppress soil-borne pathogens and pests (ALI et al., 2015; MUPAMBWA and MNKENI, 2018). While the advantages of vermicomposting are well-established, its scalability, particularly in large-scale farming systems, may be limited by operational costs, feedstock availability, and labor intensity.

2.8.7. Bio-surfactants

Microorganisms, including bacteria, yeasts, and fungi, are capable of producing surface-active biomolecules, which are frequently referred to as biosurfactants. These biosurfactants possess both hydrophilic and hydrophobic regions and exhibit amphiphilic properties. These compounds are essential for enhancing the bioavailability of hydrophobic compounds, such as hydrocarbon pollutants, heavy metals, and specific essential nutrients. They function by improving the solubilization and desorption processes, which in turn increases the availability of the surface and increases the probability of microbial uptake. Furthermore, these biomolecules regulate the adhesion and detachment of microbial cells from surfaces, which affects the dynamics of colonization in both soil and rhizosphere environments (SACHDEV and CAMEOTRA, 2013; TSIPA et al., 2021). Across a variety of domains, biosurfactants have exhibited utilities, such as the acceleration of hydrocarbon biodegradation in contaminated soils, the suppression of phytopathogens through their antifungal, antiviral, insecticidal, and anti-mycoplasma activities, and the promotion of nutrient accessibility for beneficial plant-associated microbes. Their importance in sustainable soil and plant health management strategies is emphasized by their multifunctionality. Sustainable agriculture is significantly enhanced by organic fertilizers that are derived from plant or animal sources, as they enhance soil fertility and plant development. They not only improve physical characteristics, such as soil structure, water retention capacity, and erosion resistance, but also buffer soil against pH fluctuations and suppress disease incidence. The slow nutrient release and synchronization with plant demand continue to be challenges; however, their contribution to soil biodiversity and long-term fertility render them indispensable elements in environmentally responsible crop production.

2.9.   Integrated nutrient management

In an effort to mitigate the dependence on chemical fertilizers, Integrated Nutrient Management (INM) is a sustainable agronomic strategy that strategically integrates the complementary advantages of organic and inorganic nutrient sources (CHEN, 2006). INM's success is contingent upon the safe and efficient utilization of locally available resources, the existing nutrient profile of the soil, and the nutrient requirements of the crop (DAMBALE et al., 2018a). This balance promotes the efficient utilization of nutrients, thereby enhancing soil fertility and reducing environmental degradation and nutrient losses. Through consistent nutrient availability and sustained improvements in soil health, the

primary goal of INM is to optimize crop yields (ALI et al., 1970; ELKHOLY et al., 2010; VANLAUWE et al., 2010). There are numerous studies that have confirmed the effectiveness of this approach in a variety of crops. For example, the growth and productivity of crops such as maize, rice, wheat, tomato, and apple have been increased through the integrated application of inorganic fertilizers and organic manures (ROBA, 2018), as well as soybean and wheat (BHATTACHARYYA et al., 2008). Micronutrients, in conjunction with organic and inorganic amendments, have been linked to substantial yield enhancements in oilseed crops, such as sunflower (MAHAPATRA et al., 2023). Specifically, INM has been demonstrated to improve the quality, yield, and vigor of sunflower plants while simultaneously mitigating environmental stressors. AKBARI et al. (2011a) conducted research that indicated that the optimal grain yield was achieved by combining inorganic and organic fertilizers. HELMY and RAMADAN (2009) observed improvements in morphological parameters, including plant height and leaf number, under comparable treatments. Beyond crop performance, INM has demonstrated significant improvements in soil properties, such as increased microbial biomass, improved nutrient use efficiency, and reduced reliance on synthetic fertilizers (ELKHOLY et al., 2010; ABEDI et al., 2010; ALI et al., 1970). INM also plays a significant role in environmental protection by reducing greenhouse gas emissions and enhancing nutrient cycling (PARAMESH et al., 2023). As a viable alternative to conventional high-input farming systems, the adoption of integrated nutrient management is gaining progress due to its ecological and agronomic benefits. INM is a fundamental component of sustainable agriculture (GHOLAMHOSEINI et al., 2012) due to its ability to improve soil fertility, maintain productivity, and reduce environmental impact.

2.9.1. Effect of the combination of farmyard manure and inorganic fertilizer

The integration of inorganic and organic fertilizers in sunflower cultivation has generally exhibited positive effects; however, the consistency and extent of these benefits are still under critical scrutiny. In sunflower cultivation, the combined use of various fertilizer sources has been reported to improve growth, yield, and economic return under an integrated nutrient management system (BURIRO et al., 2015b; DAMBALE et al., 2018a). DAMBALE et al. (2018a) specifically observed that the highest plant height, leaf area index (LAI) at 75 days after sowing, and total dry matter accumulation were obtained with 100% of the recommended dosage of fertilizer (RDF) plus 5 t/ha farmyard manure (FM). In the same vein, BYRAREDDY et al. (2010) discovered that the combination of FM (8–10 t/ha) and RDF (NPK at 62.5:75:62.5 kg/ha) consistently enhanced plant height, leaf number, and seed

and stalk yield over a two-year trial. These findings were further supported by NANJUNDAPPA et al. (2001), who demonstrated that the use of integrated fertilizers resulted in increased growth and yield. Nevertheless, the efficacy of integrated nutrient management is not consistent. Despite the fact that these studies emphasize the advantages of RDF and FM combinations, the scope of their findings is restricted by the lack of consensus on optimal proportions and the varying application rates. Additionally, although enhanced nutrient availability and microbial activity are frequently cited as contributing factors to favorable results (BHATTACHARYYA et al., 2008), this mechanism is not consistently quantified or validated across studies. Further, the quality of oil appears to be inconsistently improved under integrated fertilization. According to SHOGHI-KALKHORAN et al. (2013), the oil content reached its maximum with either 100% FM or a 50:50 blend of FM and NPK, while the content of linoleic and oleic acid was altered by various combinations. On the other hand, the impact of both integrated and individual fertilizer applications on oil content was negligible, as demonstrated by HELMY and RAMADAN (2009) and NANJUNDAPPA et al. (2001), highlighting the variability in outcomes. The equilibrium between vegetative response and nutrient inputs is the focus of certain investigations. For example, AKBARI et al. (2011b) underscored the potential for synergistic effects by integrating organic and chemical inputs. However, they also demonstrated that only specific ratios (e.g., 50% N + 50% FM) substantially enhanced biological and oil yield in comparison to others. In a similar vein, BOCCHI and TANO (1994) investigated the relationship between nitrogen and organic fertilizers. They determined that a 50:50 mixture of nitrogen and FM yielded superior results in seed production, biological yield, and oil yield, statistically surpassing both lower and higher FM:N ratios. ESMAEILIAN et al. (2012) observed a substantial increase in achene production in sunflower sites treated with FM alone or in combination with chemical fertilizers, which supports the notion that organic amendments can match or enhance yields when correctly integrated. The benefit of combining FM and nitrogen is still being supported by recent evidence. IRIKA (2015) discovered that the combination of 60 kg/ha N and 10 t/ha FM substantially enhanced shoot biomass, seed oil content, and leaf development. Similarly, RASOOL et al. (2013) reported that a high-input combination of nitrogen, sulfur, and FM resulted in superior LAI, dry matter accumulation, and plant height. These results indicate that the synergy between macronutrients and organic inputs may be improved through the use of multi-nutrient supplementation. However, the marginal advantages of these combinations have also been called into doubt. For example, CASSMAN and JOHNSTON (1995) discovered that the highest oil yield was achieved at 30 kg/ha nitrogen alone or in conjunction with FM, indicating that there was limited synergy. In the same vein, JANMOHAMMADI et al.

(2016a) reported a mere 17% increase in achene yield when using either NPK or 15 t/ha FM in comparison to unfertilized controls. In a subsequent investigation, JANMOHAMMADI et al. (2016b) discovered that poultry manure (PM) and NPK exhibited comparable growth and yield effects, suggesting that integration does not always provide additive benefits. Additionally, essential biological constraints manifest in integrated systems. For instance, soil microbial communities may be disrupted and the decomposition of soil organic carbon (SOC) may be suppressed if nitrogen limitation restricts microbial metabolism in soils that receive both organic and inorganic fertilizers (CUI et al., 2022). This is consistent with the findings of SINSABAUGH and FOLLSTAD SHAH (2011), who observed that crop productivity can be negatively impacted by the competition between plants and microorganisms for nitrogen in areas of nitrogen deficiency.

2.9.2. Effect of the combination of poultry manure and inorganic fertilizer

The integration of poultry manure with inorganic fertilizers has been identified as a promising approach to improve soil fertility and crop productivity by promoting a harmonious synergy between organic and chemical nutrient sources. Although the agronomic advantages are readily apparent, a more thorough investigation is necessary to determine the extent to which these combinations surpass individual applications. For instance, SAYED et al. (2022) found that sunflower growth and yield parameters were significantly enhanced by the foliar application of potassium nitrate (KNO_3) at 3%, in conjunction with 14 t/ha of poultry manure, when sunflowers were subjected to water stress. Nevertheless, the broader applicability of this study under optimal water conditions is restricted by its specific stress context. In the same vein, BURIRO et al. (2015b) discovered that the application of 6–8 t/ha of poultry manure in conjunction with 50–75% of the prescribed NPK dose resulted in an increase in plant height, stem diameter, head size, crop yield, and oil content. These findings indicate that the partial substitution of inorganic fertilizers with organic inputs has the potential to maintain or even improve productivity, thereby potentially reducing the reliance on chemical inputs. However, optimal outcomes suggest that precise dosage combinations are necessary, as no single rate is universally recommended across studies, which raises concerns about the transferability of the results to various agroecological zones. MUNIR et al. (2007) also underscored that the efficiency of fertilizer use and the yield of sunflowers were improved by supplementing NPK fertilizer (50-75-50 kg/ha) with 8 t/ha of poultry manure. The reported improvements are promising; however, the study does not investigate the economic feasibility of recurrent high-dose organic applications or the long-term health

of the soil. It is intriguing that the same study also demonstrated that goat/sheep dung and poultry manure, when applied at a rate of 6–8 t/ha, could more effectively replace 25–50% of the recommended NPK dosage than buffalo dung. This comparative analysis indicates that the decomposition dynamics and nutrient content of different manure types can have a substantial impact on the response of the crop and the availability of nutrients. Although all manure varieties can be beneficial, BURIRO et al. (2015b) advised that their efficacy is contingent upon factors such as crop type, soil conditions, and application rate. This emphasizes the significance of context-specific nutrient management, as opposed to generalized recommendations. The necessity for additional research into mineralization rates, microbial interactions, and long-term effects of various organic amendments when combined with synthetic inputs is also underscored by the inconsistent performance of different manures.

2.9.3. Effect of the combination of cattle manure and inorganic fertilizer

The synergistic effects of organic and synthetic nutrient sources have received significant attention in the integration of cattle dung with inorganic fertilizers, as it has the potential to improve soil fertility and crop yield. Although numerous studies have emphasized its efficacy, additional investigation is necessary to determine the consistency and scalability of these advantages. For example, ESMAEILIAN et al. (2012) found that the application of manure—either alone or in a 50:50 ratio with chemical fertilizers—significantly increased the yield of sunflower seeds, protein content, and oil production. The study offers limited mechanistic insight into how these combinations influence nutrient uptake efficacy or long-term soil health, despite the fact that the nutrient levels improved irrespective of the fertilization procedure. In the same vein, TABASSUM (2021) found that the application of 10 t/ha of bovine manure or 75% of the recommended inorganic fertilizer rate, either individually or in combination, enhanced sunflower yield and plant development. Nevertheless, the fact that partial substitution performed similarly to combined application raises concerns about the additive value of integration and may indicate the existence of synergistic effects, particularly if organic inputs alone can produce comparable results. The incorporation of zeolite, a hydrated aluminosilicate with exceptional water retention properties and a high cation-exchange capacity, has been investigated as a method to enhance the efficacy of manure and nutrient retention. KHODAEI JOGHAN et al. (2012) demonstrated that the use of 50% nitrogen from cattle manure and 50% from urea and zeolite resulted in a higher biomass and seed yield than non-zeolite regimens. It is important

to note that the improvement was more pronounced when zeolite was incorporated during manure decomposition rather than applied directly to the soil. This suggests that the timing and manner of application are critical for maximizing benefits. The integrative approach is further supported by the improved soil water-holding capacity and the enhanced absorption of micronutrients such as Mn, K, and Fe. GHOLAMHOSEINI et al. (2012) further stated that the crystalline structure of zeolites enables the reversible absorption of substantial amounts of water, which further enhances their utility in arid and semi-arid environmental conditions. In addition, GLISIC et al. (2009) discovered that H_2CO_3 from cattle manure could enhance humus content and neutralize soil acidity by interacting with the soil adsorption complex, suggesting that there are additional soil-conditioning effects. KHODAEI-JOGHAN et al. (2018) conducted a study that further substantiated these findings by demonstrating that the combination of zeolite and bovine manure considerably increased the productivity of sunflower seeds in both drought and well-irrigated conditions. This implies that the integration of manure and zeolite not only enhances productivity but also may contribute to long-term sustainability by reducing reliance on synthetic fertilizers. Nevertheless, the efficacy of zeolite is contingent upon factors such as soil texture, irrigation regime, and manure composition, necessitating careful calibration in these systems. In conclusion, the integration of cattle manure with inorganic fertilizers, and particularly with zeolite, provides more promising, context-dependent outcomes, despite the fact that cattle manure alone exhibits measurable agronomic benefits.

2.9.4. Effect of the combination of vermicompost and inorganic fertilizer

Vermicompost has been extensively acknowledged for its ability to enhance plant growth and yield, whether it is used exclusively or in conjunction with mineral and organic fertilizers (JAVED and PANWAR, 2013; SINGH et al., 2011). Nevertheless, a critical evaluation of its efficacy in various environmental contexts and fertilization strategies is necessary. ELANKAVI (2017b) demonstrated that the combination of 50–100% of the recommended fertilizer dose with vermicompost at 2.5 t/ha resulted in significant improvements in yield-related parameters, including seed number per head, filled grain count, and seed yield, as well as in soil quality indicators such as organic carbon, microbial activity, and nutrient availability. In an additional experiment, ELANKAVI (2017b) demonstrated that the growth and yield traits, including plant height, LAI, dry matter, head diameter, and 100-seed weight, were significantly improved by the integrated application of RDF and vermicompost at 5 t/ha, in conjunction with foliar manganese sprays at 40 and 60 days after sowing. In the same vein,

FARHAD et al. (2020) reported significant enhancements in agronomic performance, including plant height, head diameter, seed count, test weight (TKW), and net return, when chemical fertilizers were supplemented with vermicompost. These results are consistent with RAMESH (2018), who discovered that the application of pressmud-derived vermicompost at a rate of 2.5 t/ha in conjunction with 75% nitrogen resulted in an increase in sunflower growth, seed output, oil content, and oil yield. This finding underscores the potential of vermicompost as a nitrogen-efficient organic amendment. SEFAOGLU et al. (2021) also emphasized the efficacy of incorporating 1.5 t/ha vermicompost with 100 kg/ha nitrogen and 80 kg/ha phosphorus, identifying this as the most advantageous combination for seed and oil yield. It is intriguing that their findings suggested that the highest oil content was obtained with vermicompost alone, despite the fact that combined use maximized the overall yield. This difference underscores the significance of designing fertilizer strategies to meet specific production objectives, such as prioritizing oil quality over yield. This trend was further supported by SHARMA et al. (2008), who observed that the highest grain yield and TKW in sunflower were achieved when vermicompost was combined with nitrogen fertilizers. Nevertheless, the superiority of integrated approaches is not universally supported by all studies. In a study on sweet maize hybrids, LAZCANO et al. (2011) found that the yield and kernel quality of the hybrids were enhanced by the combination of vermicompost and conventional fertilizers. However, these improvements were not consistently greater than those obtained using mineral fertilizers alone. This implies that, although vermicompost provides invaluable organic matter and microbial stimulation, its advantages may be context-dependent and not always synergistic when used in conjunction with chemical fertilizers.

2.9.5. Effect of the combination of compost and inorganic fertilizer

Particularly in the context of integrated nutrient management systems, the agronomic value of compost is frequently compared to that of manure. The potential to enhance crop productivity, improve soil fertility, and promote sustainable agricultural practices has generated significant interest in the combination of organic and inorganic fertilizers. KUMAR et al. (2017) observed that the highest accumulation of dry matter in the stem and capitulum at harvest was achieved when RDF (Recommended Dose of Fertilizer) was combined with compost at a rate of 5 t/ha. This observation was made in this context. Compost's capacity to positively impact biomass partitioning and plant development is underscored by this discovery. Notwithstanding, the advantages of compost integration necessitate careful assessment. AHMAD et al. (2013) discovered that the yields were

higher when 50% of the recommended NPK dose was used in conjunction with organic sources than when 50% NPK was used alone. However, these yields were still inferior to those obtained with the full (100%) NPK application. Organic amendments can improve soil structure and microbial activity and enhance nutrient use efficiency. However, they may not always completely substitute the yield effects of complete mineral fertilization under certain conditions. This outcome emphasizes a critical limitation. BRAR et al. (2015) similarly propose that, although integrated nutrient strategies are beneficial for the long-term health and sustainability of soil, the yield enhancements that result from the partial replacement of chemical fertilizers with compost may not always satisfy the requirements of high-yielding cropping systems. This discrepancy underscores the necessity of context-specific approaches that balance the ecological advantages of decomposition against potential trade-offs in crop productivity.

2.9.6. Effect of the combination of organic, inorganic, and biofertilizer

Mycorrhizal fungi and rhizobacteria are microorganisms that are increasingly acknowledged for their contributions to plant health and nutrient cycling when employed as biofertilizers (PARAMESH et al., 2023). Integrated nutrient management strategies that integrate biofertilizers with organic and inorganic inputs have demonstrated substantial advantages in terms of soil fertility and crop yield. For example, MUKHERJEE et al. (2019) and RAJ and MALLICK (2017) have shown that the combination of 50% RDF with poultry manure and biofertilizers (such as phosphate-solubilizing bacteria and Azotobacter) improves the oil content, seed yield, dry matter accumulation, and leaf area index of sunflowers. In the same vein, MALLICK and MAJUMDER (2022) reported that the integration of 50% RDF, vermicompost (2.5 t/ha), biofertilizers, and foliar applications significantly enhanced nutrient availability and seed yield. NANJUNDAPPA et al. (2001) also emphasized that yield enhancements were observed when farmyard manure and biofertilizers were used in conjunction with the recommended fertilizers. Nevertheless, the outcomes were inconsistent: EM (Effective Microorganisms) increased shoot biomass and crop yield in the presence of NPK and FYM treatments, but it decreased yield by 23% in the presence of green manure treatments and suppressed nodulation in all organic amendments except NPK (JAVAID and BAJWA, 2011). The context-dependent nature of EM performance is underscored by these findings. The potential of the synergistic use of EM and organic inputs extends beyond sunflowers. KHALIQ et al. (2006) reported a 44% increase in cotton yield and economic efficacy when EM was employed with half the RDF and organic materials.

Similarly, YOUNAS et al. (2022) underscored the environmentally safe function of EM in enhancing the quality and yield of wheat, although the effects are contingent upon the type of amendment. However, certain combinations are unable to satisfy the nutrient requirements of crops. SANASAM and GEETHA (2016) discovered that sunflower growth could not be sustained by vermicompost or 50% RDF plus *Phosphobacterin* alone. Conversely, NAMVAR et al. (2012) demonstrated that the application of 200 kg/ha nitrogen in conjunction with biofertilizers resulted in the most favorable grain parameters and growth. Additionally, CHOUDHARY et al. (2017) noted that the vigor of plants was substantially enhanced by the use of sulfur (45 kg/ha) in conjunction with sulfur-oxidizing microorganisms. The efficacy of combining RDF with *Azospirillum* and *Phosphobacterium* to improve sunflower performance was substantiated by REDDY et al. (2005). Nevertheless, the added utility of combining biofertilizers with organics or inorganics was contested by other studies. In infertile soils, KHALIL et al. (2004) observed that the addition of manures resulted in an increase in wheat biomass and nutrient absorption, irrespective of the presence of biofertilizer or not. In the same vein, ALZAMEL et al. (2022) observed that the oleic acid content was adversely affected by the compost with biofertilizers, despite the fact that it increased the yield of seeds and oils. This underscores the potential impact of fertilizer combinations on crop quality characteristics. In summary, the efficacy of sunflower growth and yield optimization is contingent upon the specific crop, soil, and environmental conditions, despite the potential benefits of the integrated use of organic, inorganic, and microbial inputs. Therefore, it is imperative to customize combinations to align with desired outcomes, including sustainability, quality, or yield, in order to ensure effective nutrient management.

2.9.7. Effect of the combination of macronutrients, micronutrients, and organic manure

The integration of organic manures, micronutrients, and macronutrients has garnered significant attention due to its role in enhancing soil fertility and improving crop productivity. The highest plant height, stem girth, head diameter, seed number per head, seed weight, seed index, and overall seed yield were attained when NPK (90-45-45 kg/ha) was combined with Zn-B (15-1.5 kg/ha), with nitrogen used through fertigation, according to a study conducted by HUSSAIN SIDDIQUI et al. (2009). In the same vein, PATTANAYAK et al. (2016) found that the application of RDF with either 1 kg/ha boron or 25 kg/ha zinc sulfate ($ZnSO_4$) significantly increased stem girth at multiple growth stages (30, 45, 60, 75 DAS, and harvest), indicating that both micronutrients contributed similarly to

structural development. The agronomic benefits of combining micronutrients with organic and inorganic fertilizers were further underscored by POPOOLA et al. (2016), who discovered that such combinations consistently yielded favorable results. The application of 100% NPK, 5 t/ha farmyard manure, and 20 kg/ha of $ZnSO_4$ and $FeSO_4$ significantly enhanced sunflower growth parameters, including plant height, functional leaf count, and dry matter accumulation, as reported by WAGHMARE et al. (2022). The synergistic effects of macro- and micronutrient combinations were also emphasized by the significant increases in head diameter, seed yield, and oil yield that resulted from this treatment. JANMOHAMMADI et al. (2016b) emphasized that the integration of organic manure with nano-chelated micronutrients was particularly effective in maximizing sunflower yield in semi-arid environments. In the same vein, KHAN et al. (2022) reported significant enhancements in sunflower growth, yield, and oil content as a result of the combined application of sulfur and poultry manure. This underscores the importance of combining organic amendments with essential secondary nutrients. In general, Integrated Nutrient Management (INM) has been observed to be effective in improving soil health, nutrient availability, and absorption efficiency by strategically combining organic manures (e.g., farmyard, poultry, cattle manures, compost, vermicompost) with inorganic fertilizers (e.g., N, P, K). Inorganic N and K can be applied at rates of 50–90 kg/ha, while organic manure rates typically fall within the range of 5 to 15 t/ha. The benefits of INM are further enhanced by the incorporation of micronutrients and biofertilizers, which contribute to sustainable agriculture, particularly in the face of adverse conditions like drought. Nevertheless, the necessity of localized nutrient management strategies is underscored by the potential for outcomes to differ based on the specific nutrient formulations, soil types, and climatic conditions.

3. 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 (KHAEIM et al., 2022a, 2022b). 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 crops 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.

3.1. 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), 47°35'37"N, 19°21'55" E. 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).

3.2. 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 on a daily basis at each temperature. The germination parameters were explained according to KENDE et al. (2024). It employed essential measurements derived from the Four-parameter Hill function as outlined by El-KASSABY et al. (2008).

$$y = y_0 + \frac{ax^b}{x^b + c^b} \quad (1)$$

The partial derivative of the four-parameter Hill function was used to determine the time to maximum germination rate (TMGR):

$$TMGR = \sqrt[b]{\frac{c^b(b-1)}{b+1}} \quad (2)$$

In this context, y denotes the cumulative germination% at time x, y_0 signifies the y-axis intercept, a indicates the asymptote, b is a parameter that determines the shape and steepness of the germination curve, and c represents the half-maximal activation level. We computed the t_{50} value, which reflects the duration at which 50% of the seeds have germinated, serving as a crucial parameter for evaluating germination rates under varying conditions, as outlined by FARROQ et al. (2005)

$$T_{50} = T_i + \left(\frac{\frac{N}{2} - N}{N_j - N_i} \right) * (T_j - T_i) \quad (3)$$

In this context, t50 denotes the duration required to achieve 50% of the final or maximum germination, N represents the final number of germinated seeds, and Ni and Nj signify the cumulative numbers of seeds that have germinated at times T_i and T_j, respectively, with the condition Ni < N/2 < Nj. We calculated the mean germination time (MGT) as per LABOURIAU, 1983), which signifies the rapidity with which the majority of seeds in a batch germinate as follows:

$$MGT = \frac{\Sigma(N_i * N_j)}{N_i} \quad (4)$$

where N_i represents the number of seeds that have germinated at time T_i. The uniformity of germination (U) reflects the disparity in duration between the highest and minimum germination

durations, signifying the consistency of the germination process. We computed it in the following manner:

$$U = t_{max} - t_{min} \quad (5)$$

t_{max} is the time at which the highest proportion of seeds has germinated, whereas t_{min} signifies the time at which the least percentage of seeds has germinated.

The area under the curve (AUC) for the germination rate was determined using the Four-parameter hill function as referenced in (EL-KASSABY et al., 2008). The Germination Energy Index (GEI) of Timsons, as determined by (TIMSON, 1965), was computed as follows:

$$GEI = \sum k = \sum_{i=1}^{k} G_i \quad (6)$$

where G_i represents the cumulative germination percentage during time interval i, and k denotes the total number of time periods. The synchronization index (E) as per (TIMSON, 1965) was generated to offer a thorough evaluation of germination performance:

$$E = -\sum_{i=1}^{k} f_i \log_2 f_i \quad (7)$$

represents the relative frequency of germination, N_i is the number of seeds that germinated at the ith time interval, and k signifies the total number of time intervals.

3.3. 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.

3.4. 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 base of the TKW as a percentage (annex). 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 (7) was used to compute the amount of water based on TKW (KHAEIM et al., 2022a, 2022b)

$$\text{TKW} * \text{Seed number}/100{,}000 = 1\% \text{ of the proposed water amount (7)}$$

The TKW of the sunflower seeds was 50.46 g. Additional information on calculating water quantity based on TKW is available in previous works (KHAEIM et al., 2022a, 2022b). 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).

Figure 2. Photos of germinated seeds after 10 days

3.5. Antifungal 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 Amistar Xtra contains two active ingredients: Azoxystrobin and Cyproconazole. This combination provides

both preventative and curative activity against a broad range of fungal diseases. 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 10% sodium hypochlorite (NaClO) is commonly used in breeding laboratories to sterilize seeds. 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.

3.6. 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).

3.7. Field Production Trials

3.7.1. Site description

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, shown in Figure 1.
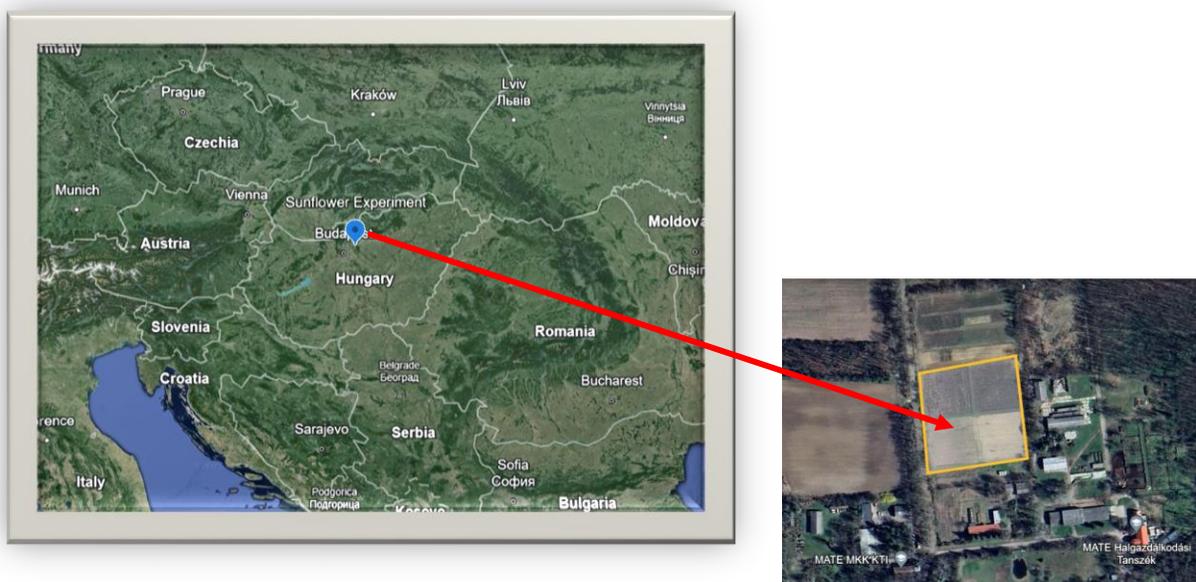
Figure 3. The experimental field viewed by satellite on 12-6-2024, Gödöllö, Hungary

The soil type of the experimental field was sand-based brown forest soil (Chromic Luvisol). The textural classification of the soil was a sandy loam with parameters shown in Figure 14. The agronomic characteristic of the soil was neutral sandy soil with variable clay content. The soil structure was susceptible to compaction issues. The water retention characteristics were poor due to the high sand content. The soil was exposed to the impacts of drought.

Table 2. Soil type of the experimental field at Hungarian University of Agriculture and Life Sciences, Crop Production Institute, Gödöllö, Hungary

	Humus %	pH (H$_2$O)	K$_A$	Sand %	Silt %	Clay %	CaCO$_3$
Soil Medium	1.32	7.08	40	49	25	26	0

3.7.2. Meteorological properties of the experimental fields

The atmospheric data, which is fundamental for comprehending diverse environmental variables, was scrupulously obtained through the deployment of a rigorously calibrated meteorological tower that was specifically engineered to acquire and document information at exact intervals of every 10 minutes, and this tower was strategically and ideally situated within the confines of the experimental site itself, thereby guaranteeing optimal conditions for data collection.

3.7.3. Precipitation of the experimental period
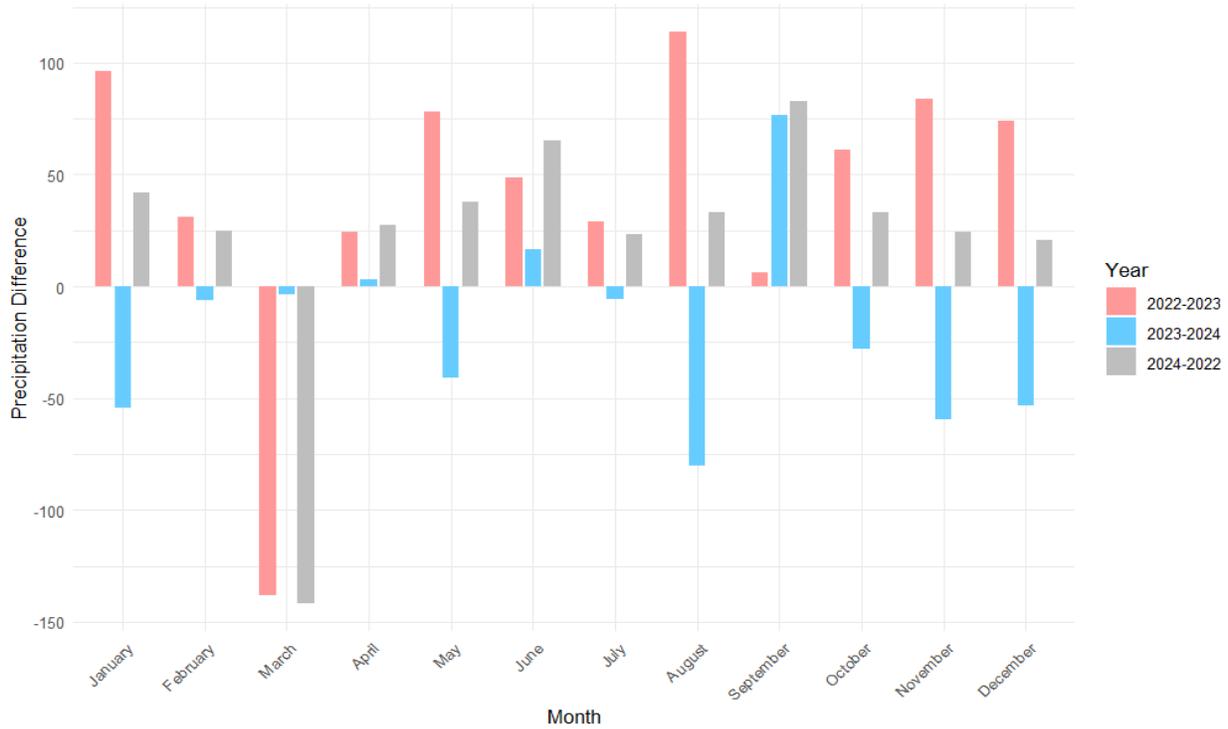
Figure 4. Monthly precipitation (mm) data in the experiment area during the three seasons 2022-2024

The data above illustrates the precipitation differences between the periods 2023-2022 and 2024-2023. A comparison of the years 2022-2023 reveals a noticeable increase in precipitation levels in 2023 compared to 2022 (Figure 4). Specifically, during May, which corresponds to the vegetative phase of sunflower growth, precipitation rose by 78.29 mm. In August, a critical period aligning with the seed-filling stage, precipitation increased by 113.69 mm. In contrast, the 2023-2024 period shows a negative difference, with 2023 recording higher precipitation levels than 2024 throughout the year. The most significant decreases were observed in May and August, with reductions of 40 mm and 80.40 mm, respectively. The graphical representation highlights considerable disparities between the three years, particularly in May and August, key stages for sunflower growth. Notably, 2022 was the driest year among the three.

3.7.4. Temperature of the experimental period
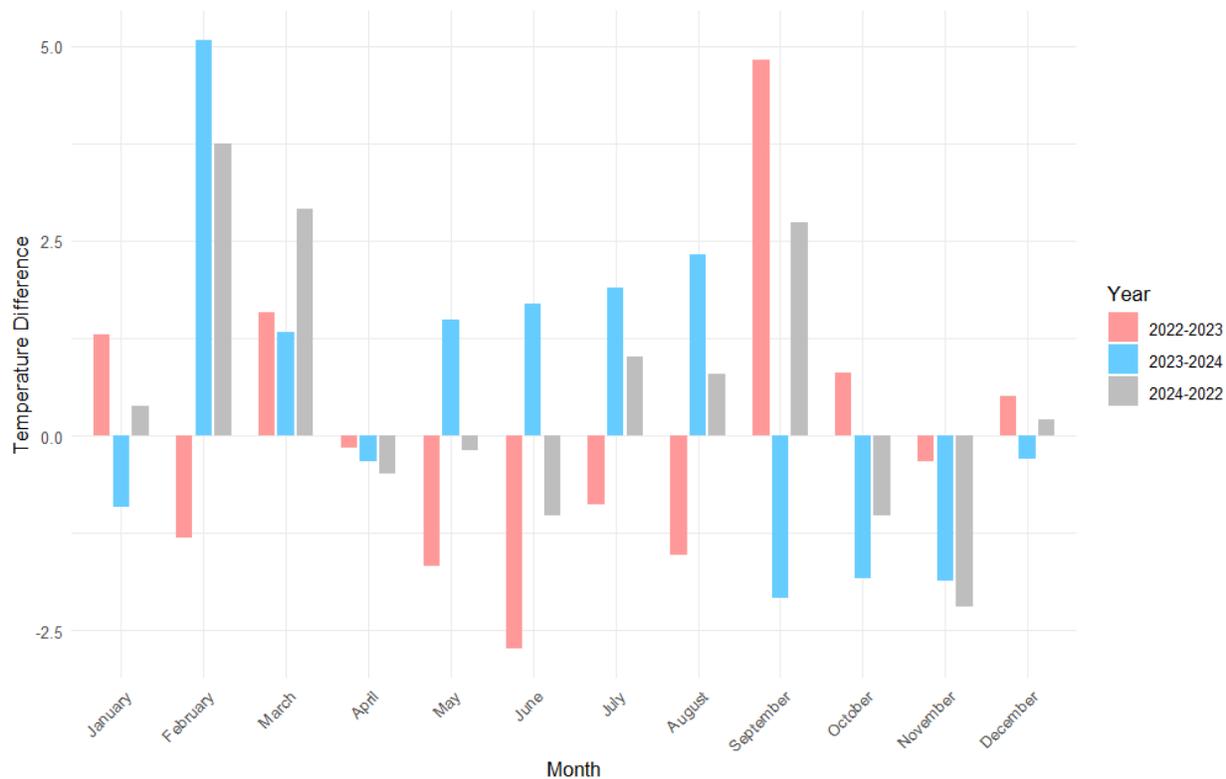
Figure 5. Monthly recording of Air temperature (°C) data in the experiment area during the three seasons 2022-2024

The highest temperatures in 2022 were recorded in June and August (Figure 5). In comparison between 2023 and 2024, temperatures were generally higher in 2024, especially during the sunflower crop's growth period. For the period between 2024 and 2022, temperatures tended to be lower from April to June but higher from July to September. The highest temperatures were recorded in 2024, both from April to June and from July to September.

3.8. Agronomic Practices on Field Trials

This section provides a detailed account of the experimental procedures, including soil preparation, planting methods, plant protection measures, application of treatments, and techniques used for data collection.

3.8.1. Soil preparation

The soil preparation involved plowing as primary tillage to a depth of approximately 30 cm using a moldboard plow. This was followed by harrowing to level and close the surface. Seedbed preparation was conducted with a shallow tine cultivator. These steps ensured a fine seedbed suitable for optimal sunflower root development and water infiltration.

3.8.2. Planting

The sunflower was sown into a 1ha field with four 2500 m^2 parcels, where we used the crop rotation sequence of Wheat -> sunflower -> maize-> green manure (soybean). Seeds were sown directly into the prepared seedbed at a depth of 3 cm, with a spacing of 35 cm between seeds, with a MaterMacc MS4100 planting machine. Rows were spaced 75 cm apart to ensure adequate air circulation and sunlight exposure. The planting was timed to coincide with soil temperatures consistently above 15 °C before rain events to promote germination and seedling vigor. The sunflower plants were grown under rainfed conditions throughout the year. The table below highlights the date of each phase of Sunflower, Fertilizer Treatment, and Harvest time for each year.

Table 3. Timeline of Sowing, Treatment Application, and Key Growth Stages for the Years 2022-2024

Year	Sowing time	Treatment application	Vegetative time	Blooming time	Seed filling starting	Harvest
2022	5th May	10th June	25th May	11th July	2nd August	4th October
2023	25th April	29th June	20th May	17th July	31th July	12th September
2024	8th May	20th June	26th May	8th July	30th July	19th September

3.8.3. Plant protection

Weed control was applied before plantation as preemergence with s-metolachlor and selective monocotyledon with quizalofop-P-ethyl at the 4-6 leaf stage of the sunflower. The plant protection was the same throughout the 3 years of the research. The sunflowers' heads were covered, beginning with the seed loading process, to prevent the loss of seeds due to avian consumption.

3.8.4. 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 split applications of nitrogen from dried pelleted cattle manure and ammonium nitrate, and potassium (K) from sulfate 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 (Figure 6). 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).

Dried pelleted cattle manure (organic fertilizer) 6%N	Effective microorganism **EM1**	Sulphate of Potash 50% K_2O	Ammonium nitrate **34%N** (inorganic fertilizer)

Figure 6. Photos of fertilizers used in the experiment

The study employed a Randomized Block Design (RBD) with three blocks, each containing seven treatments (including control), to systematically evaluate treatment effects on sunflower growth and yield parameters (Table 4). This factorial design with replication ensured robust statistical analysis while accounting for potential field variability. The treatment structure comprised:

- **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 (Dried pelleted cattle manure) and 50% inorganic manure (Ammonium nitrate)
- **EM-1:** Effective micro-organismes 40 l/ha (EM1)
- **K+GOIM**: a combination of K and GOIM
- **K+EM-1**: a combination of K and EM-1**.**
- **GOIM+EM:** a combination of GOIM and EM

Table 4. Plan of the layout of the experimental site

CONTROL	K+EM-1	GOIM
POTASSIUM K	GOIM	GOIM+EM-1
GOIM	EM-1	K+GOIM
EM-1	POTASSIUM K	CONTROL
K+GOIM	CONTROL	K+EM-1
K+EM-1	GOIM+EM-1	EM-1
GOIM+EM-1	K+GOIM	POTASSIUM K

3.9. Details of the collection of experimental data

Three plants from each net 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 diameter (girth), 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 (MATE). The quality parameters (oil content, protein, water content) and Fatty acid profile were determined in MATE Központi Vizsgálólaboratórium in Kaposvar.

3.9.1. SPAD number

The SPAD readings, non-destructive, provide a rapid estimation of the chlorophyll content. Chlorophyll content was estimated using a SPAD-502 Plus Chlorophyll Meter (Konica Minolta, Japan). The SPAD meter measures the absorbance of the leaf at two wavelengths (650 nm and 940 nm) to estimate chlorophyll content. Measurements were taken between 10:00 and 12:00, in fully

expanded, healthy leaves from the middle section of each plant were selected for measurement. A total of 9 plants per treatment group were sampled.

Figure 7. Collecting data using the SPAD device

3.9.2. Leaf Area Index

Leaf Area Index (LAI) was measured to assess the canopy structure and density of the plant community. LAI provides a quantitative measure of leaf area relative to the ground surface area, which is essential for understanding light interception, photosynthetic capacity, and overall plant productivity. LAI was measured using the LAI-2200C Plant Canopy Analyzer (LI-COR Biosciences, Lincoln, NE, USA). This instrument estimates LAI by analyzing the light attenuation through the canopy using fisheye optical sensors. Measurements were taken at three randomly selected locations within each plot. Measurements were taken early in the morning or late in the afternoon to minimize the impact of direct sunlight, which can affect the accuracy of the readings. The sensor was positioned at ground level, and five readings were taken at each sampling point to calculate an average LAI value.

Figure 8. Leaf Area Index used in the experiment

3.9.3. Plant height

The height of the sunflower head is considered the distance between the center of the tagged sunflower and the Earth's surface. The height of each sunflower head was measured using a flexible meter with a 500 cm scale and an accuracy of 1 mm.
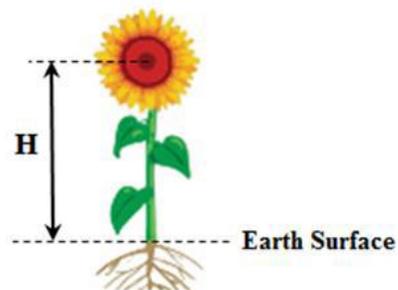
Figure 9. Measurement of Plant Height Using a Flexible Meter. (Hossein Mirzabe et al., 2016)

52

3.9.4. Number of leaves per plant

The total numbers of green leaves produced per plant were counted from three randomly selected (tagged) plants, and their average was taken as the number of green leaves per plant.

3.9.5. Stem girth

Stem girth or stem diameter was measured to evaluate plant growth and development of sunflower plants (cm). Measurements were conducted at the early flowering stage to ensure consistency. Nine plants were randomly selected from each treatment group within the experimental plots. The stem diameter was measured 10 cm above the soil surface to avoid basal swelling. A digital caliper was used to ensure precise and accurate measurements.

Figure 10. Measurement of stem girth using a digital caliper

3.9.6. Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence was measured using a FluorPen chlorophyll fluorometer (FluorPen FP 110).

Figure 11. Measurement of Quantum Efficiency of photosystem PSII using FluorPen device

FluroPen is a non-invasive indicator of photosynthetic activity and efficiency. It was measured between 8-10 am, 30 min after placing the clips on the third well-developed leaf under the sunflower head on 9 plants per treatment. The leaf is considered well-developed if larger than 4 cm. The leaves were exposed to a pulse of saturating red light of 3200 µmol/m2 /s. This device measures the Quantum Efficiency (Qy) of photosystem PSII.

3.9.7. Harvesting and threshing

The crop was harvested between the end of September and the beginning of October for each year (Table 3). 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.

Figure 12. Photos showing the Planting and Harvesting of sunflowers

3.9.8. Yield Parameters

The yield attributes of sunflower (*Helianthus annuus*) were evaluated to assess the productivity and quality of various plants subjected to varying levels of fertilizers. The parameters determined were as follows:

- Head Diameter: The diameter of the head was measured in two diagonal directions, and the average was taken as the diameter of the head in centimeters.

- Number of Seeds per Head: Seeds from 10 heads per plot were counted manually to determine the average number of seeds per head.

- TKW: Three samples of 1000 seeds from each plot were weighed using a precision balance to determine the average TKW.

- Seed Yield: Sunflower heads were harvested at physiological maturity. Seeds were threshed, cleaned, and weighed to determine the yield per hectare.

- Quality parameters: The oil content was determined using FALC BE6 Soxhlet extraction apparatus, seed moisture content using Memmert UFE 500 drying oven, and protein

percentage based to Kjeldahl method, using a Foss Tecator Digestor Auto system and Foss Kjeltec 8400 analyzer).

- Fatty Acid Profile: The percentages of free fatty acids were determined following the AOAC method 969.33 (AOAC International, 2000) using gas chromatography-mass spectrometry (Shimadzu 2010 GC) to determine the fatty acid profile of sunflowers obtained by green processes.

3.10. Statistical analysis

3.10.1. Statistical methods on germination experiments

The effects of the water level, seed number, and antifungal treatment on the germination percentage and seedling growth were analyzed and a sigmoid curve model was applied using statistical computing programs (J.M.P. Pro 13.2.1 of S.A.S. by SAS Institute, Canberra, the USA, and MS Excel 365) to fit the data and plot the best-fit temperature levels. For the data normality verifications, Kolmogorov–Smirnov and Shapiro–Wilk tests were conducted using IBM SPSS V27 (New York, NY, USA), and an analysis of variance (ANOVA) and Fisher's test of least significant differences (LSDs) were conducted used the (GenStat twelfth edition, GenStat Procedure Library Release PL20.1m, and MS Excel 365), IBM SPSS V27 (New York, NY, USA). Where the normality assumption was not reached, we used non-parametric tests such as Kruskal–Wallis with Bonferroni's post hoc test and the Mann–Whitney U test for pairwise comparisons. Germination indices were calculated, and fitting curves were created with the help of RStudio V2024.04.2 (RSTUDIO TEAM, 2024) software using several R packages for data analysis and visualization, including 'germinationmetrics', 'dplyr', 'tidyr', 'ggplot2', and 'patchwork'.

3.10.2. Statistical methods on field experiments

For statistical analysis, we used the IBM SPSS V27 (New York, NY, USA) and RStudio V2024.04.2 (RSTUDIO TEAM, 2024) software with the help of several R packages for data analysis and visualization, including RcmdrMisc; Nortest; Car; Data.Table ; Rstatix ; Ggplot2; Plyr; Pheatmap; agricolea. The effects of different fertilizer treatments and two years on growth and yield attributes of Sunflower plants were analyzed by multivariate two-way ANOVA and robust ANOVA (Welch) ANOVA when the normality was violated. The normality of the residuals was checked by Shapiro-

Wilk's test. The homogeneity of variances was checked for both factors, fertilizers and year, by Levene's test. Pairwise comparisons were performed by Games-Howell's post hoc test. We compared the effect of Year in Fertilizers groups, and we also made comparisons for fertilizers' effect in each year group, separately, to avoid the biasing effect of the potential interaction effect. Heatmaps and AMMI (Additive Main Effects and Multiplicative Interaction) PCA were performed using RStudio for visualizing the interaction patterns between treatments and to explore the relationship between fertilizer types and year factors. These methods allowed for a robust interpretation of the data by highlighting both main and interaction effects on yield and growth parameters, contributing to the understanding of how environmental and agronomic variables influence sunflower performance.

4. RESULTS

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.

4.1. Results of Germination tests

This part of the study highlights the findings of the germination test, germination response to different levels of temperature and water, optimization of seed number, and antifungal growth control to grow sunflower seeds in vitro. The germination performance of sunflower seeds varied significantly across the temperature range from 5°C to 40°C, revealing key insights into optimal temperature conditions for germination speed and success. At 5°C, the seeds showed delayed and slow germination, with a maximum germination rate of only 13.33% (Figure 13). The Time to maximum germination (TMGR) was about 12.9 days, reflecting the exact period for seeds to reach their maximum germination. The mean germination time (MGT) was 8.98 days, the longest among all tested temperatures, and the area under the curve (AUC) was particularly low (14.85), further confirming the poor and delayed germination at this low temperature. The time to reach 50% germination (T50) was 12.96 days, reinforcing the conclusion that germination at this temperature was sluggish and suboptimal. These results suggest that 5°C represents the lower limit of sunflower germination, where physiological processes are slowed significantly, likely due to suboptimal enzyme activity and reduced metabolic rates. As the temperature increased to 10°C (Figure 13), germination improved substantially. The maximum germination rate increased to 53%, and other indicators such as TMGR (8.96 days), MGT (8.75 days), and T50 (9.42 days) reflected quicker germination than at 5°C. The AUC was much higher at 233.776, indicating a significant improvement in germination success. Although germination was faster than at 5°C, the overall performance was still relatively slow compared to higher temperatures, suggesting that 10°C is still below the optimal temperature for sunflower germination.
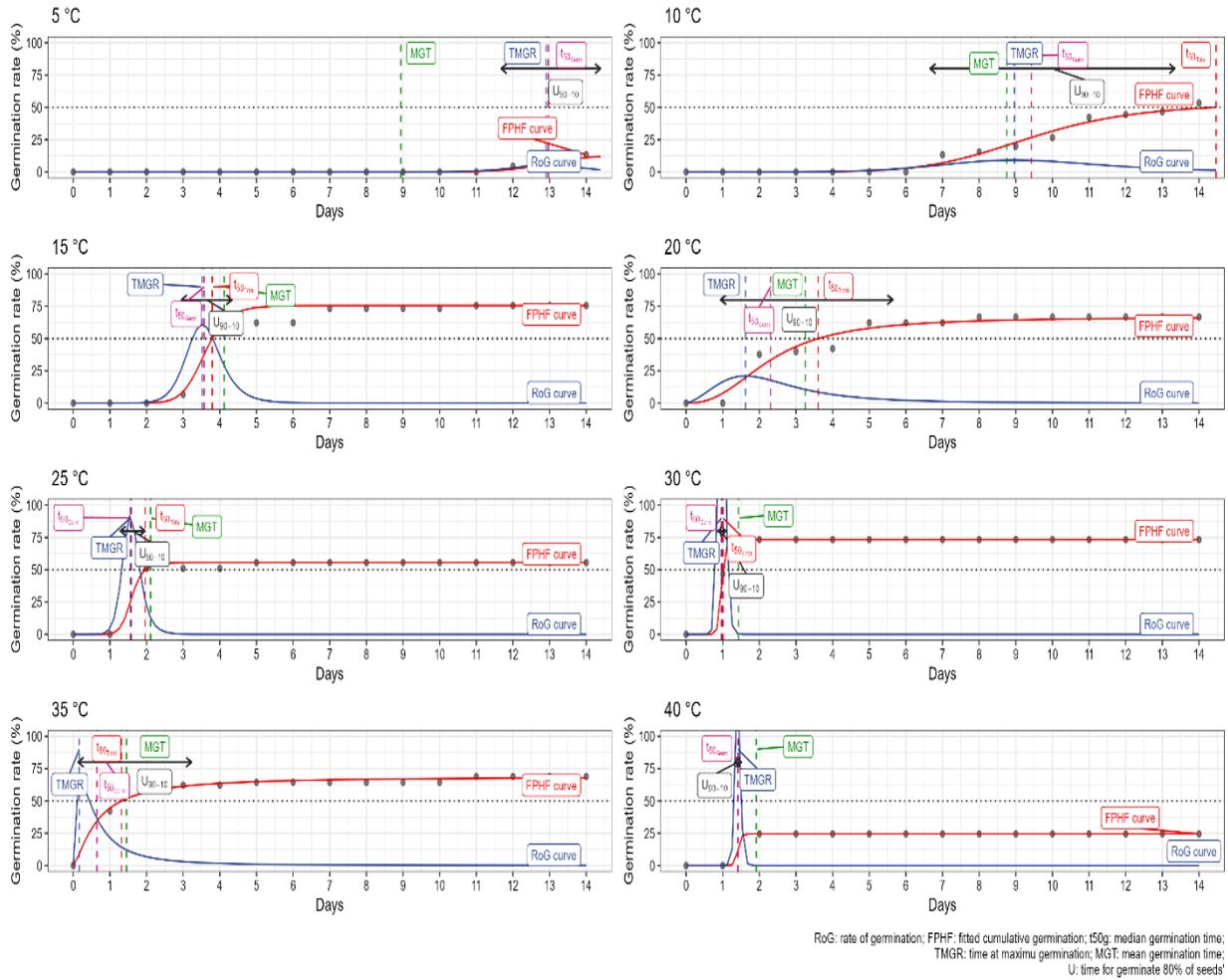
Figure 13. Germination rate at different temperature levels

At 15°C (Figure 13), sunflower seeds showed their highest germination rate of 75%. This temperature appears to represent a critical threshold, where the physiological processes governing germination become highly efficient. Germination started on the second day, with a time to maximum germination (TMGR) of 3.52 days, indicating a substantial acceleration in germination speed compared to lower temperatures. The AUC was recorded at 784.219, which further supports the efficiency of germination at this temperature. The shorter MGT (4.12 days) and T50 (3.52 days) highlight that 15°C is conducive to both rapid and successful germination, making it a potential optimal temperature for maximizing the germination rate. At 20°C (Figure 13), while the germination rate decreased slightly to 66%, other parameters, such as the time to germination and MGT (3.25), continued to improve. The seeds germinated from the first day, and the TMGR dropped to 1.62 days, while the T50 was 0.943 days. The AUC remained high at 735.58, but lower than at 15°C, reflecting a somewhat less favorable outcome. This suggests that although germination speed increases at 20°C, the maximum germination

59

rate begins to decline slightly, possibly indicating that some physiological stress might begin to affect the seeds at this temperature. At 25°C (Figure 13), the germination rate dropped further to 55.55%, with TMGR and T50 values both around 1.56 days, indicating fast germination, though not as high in efficiency as 15°C or 20°C. The MGT was shorter (2.11 days), and the AUC remained high at 688.29. This suggests that while sunflower seeds still germinate relatively quickly at this temperature, the overall germination success begins to diminish, potentially due to the temperature approaching the upper limits of the optimal range. At 30°C (Figure 13), a striking balance between speed and germination success is observed. Although the maximum germination rate was 73%, slightly lower than at 15°C, the TMGR (0.96 days) and T50 (0.97 days) were the shortest recorded, meaning that germination was the fastest at this temperature. The MGT was 1.43 days, further indicating rapid germination, and the AUC reached its highest value of 955.096, demonstrating that the overall germination process was highly efficient and consistent at 30°C. These results suggest that 30°C is an excellent temperature for sunflower germination when considering both speed and germination success. This temperature allows for rapid seedling establishment, which is advantageous for agricultural or ecological applications where fast crop development is desired. At 35°C (Figure 13), while the germination rate was still high at 68%, the AUC was lower (866.5031) than at 30°C, and the TMGR (0.163 days), MGT (1.45), and T50 (0.644 days) were among the shortest recorded. Despite this rapid germination, the slight decline in maximum germination rate and the AUC indicates that 35°C might induce some physiological stress, potentially limiting the maximum germination success even though the process remains very fast. At 40°C (Figure 13), germination speed remained high, with TMGR (1.41 days), MGT (1.91), and T50 (1.41 days) still showing fast results, but the maximum germination rate declined to 24%, and the AUC dropped sharply to 307.62. This suggests that at 40°C, sunflower seeds may be subjected to thermal stress, leading to reduced overall germination success despite the relatively fast germination observed. The optimal temperature range for sunflower germination appears to fall between 15°C and 30°C. Within this range, sunflower seeds demonstrated both high germination rates and fast germination speeds. At 15°C, the highest maximum germination rate of 75% was recorded, indicating strong germination success. As the temperature increased, germination rates remained relatively high, with 66% at 20°C and 73% at 30°C, while the mean germination time (MGT) and time to maximum germination (TMGR) decreased, showing that seeds germinated more quickly. The AUC (Area Under the Curve), which reflects overall germination efficiency, was highest at 30°C (955.096), suggesting that this temperature is particularly effective for both rapid and successful germination. While temperatures below 15°C resulted in slower germination

and reduced success, and temperatures above 30°C (such as 35°C and 40°C) led to faster germination but lower germination rates, the interval between 15°C and 30°C offers the most favorable conditions. Therefore, this range provides an optimal balance, where sunflower seeds not only germinate quickly but also achieve high germination rates, making it ideal for effective seedling establishment.

4.1.1. Effect of Temperature on seed germination and seedling growth

The time course of the germination of sunflower was conducted at temperatures ranging from 5 to 40 °C, with 5 °C intervals (Figure 14). Obvious germination was detected after the experiment began for two days at temperatures of 25 and 30°C, three days at 20 °C, four days at 15 °C, and six days at 35 °C. Sunflower seeds can germinate at temperatures between 5 and 35 °C, but the highest germination rate was observed at 25 °C.
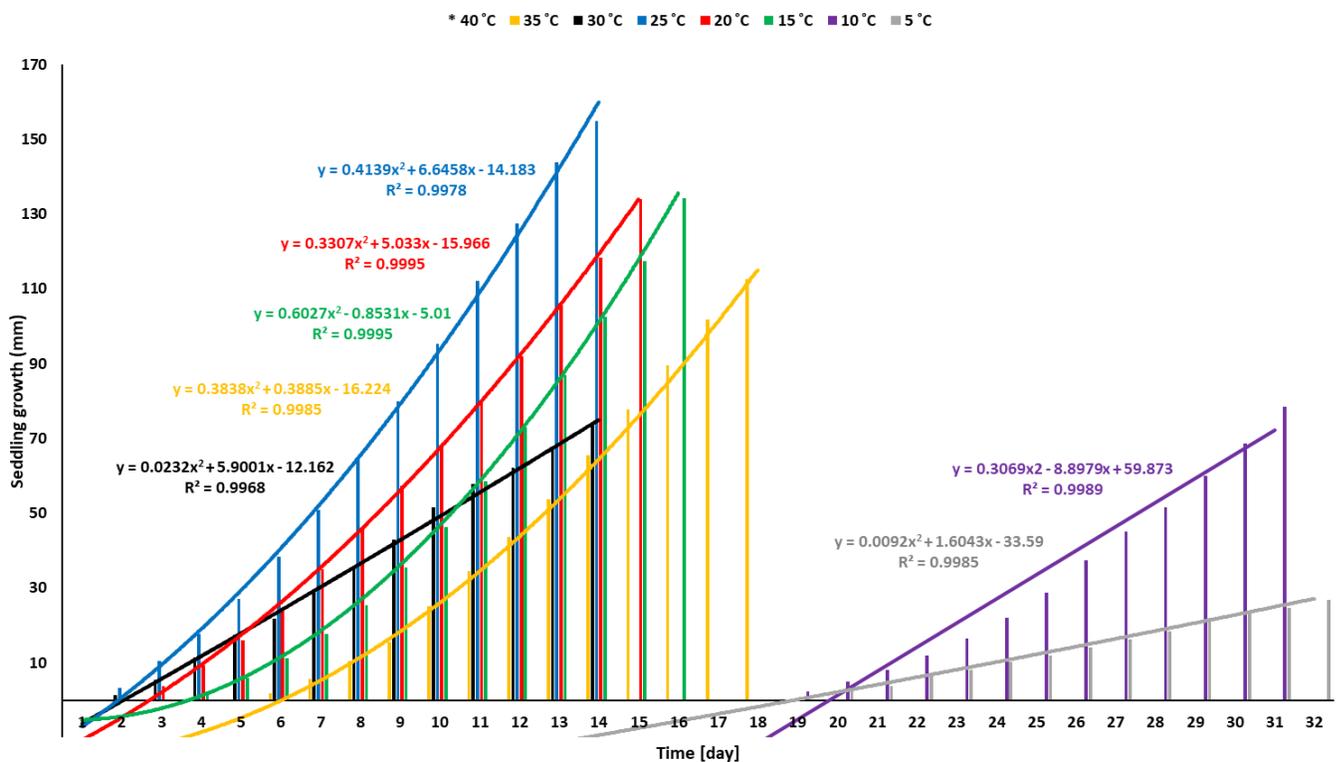
Figure 14. Germination and seedling growth of sunflower seeds vs time at different levels of temperature

Early germination occurred at 20°C and 25°C, but at 20°C, the growth rate was lower. The same pattern was observed at 15 and 20 °C; however, the germination was earlier at 20 °C. Sunflower seeds took longer to germinate at low temperatures, approximately 19 days at temperatures of 5 and 10 °C.

The minimum temperature of germination ranged between 5 and 10 °C, while the maximum ranged between 35 and 40 °C. The seeds failed to germinate at 40 degrees Celsius. Their position on the growth curve is hence zero.
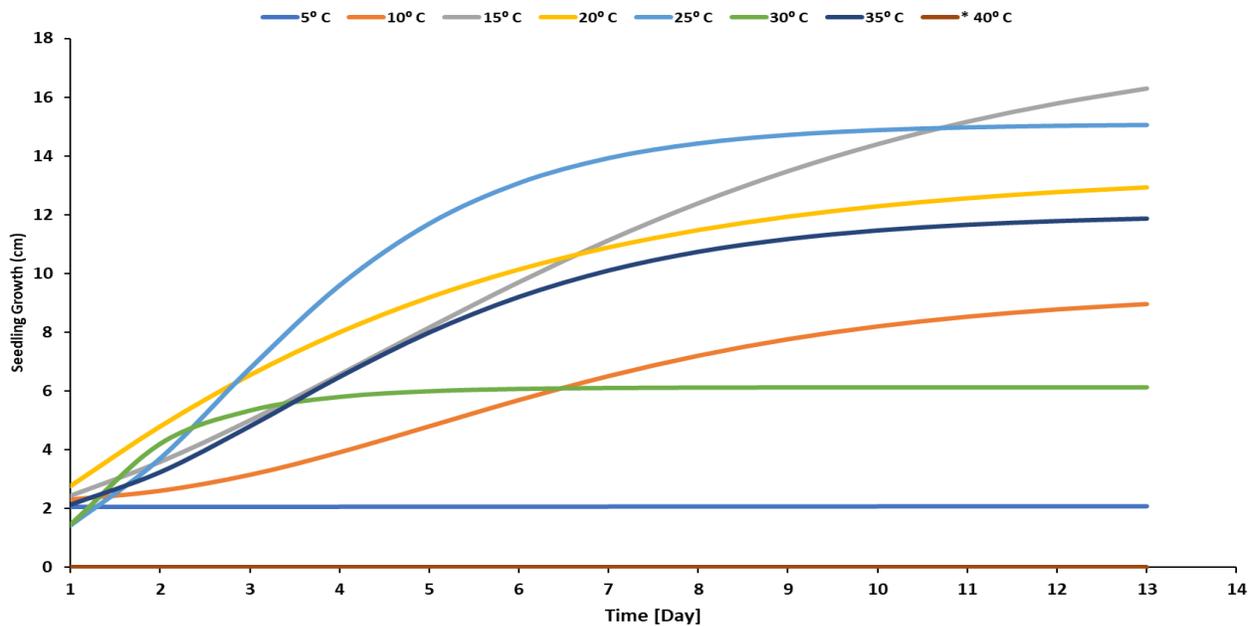
Figure 15. Effect of temperature levels on seedling growth response

Figure 15 illustrates the biological effects of different temperatures on the development of the seedling. The effect of different levels of temperature on radicles and plumules is available in the publications (HAJ SGHAIER et al., 2023). The results indicated that growth was highest at 25 °C, followed by 15 °C, 20 °C, and 35 °C. Therefore, temperatures of 25 degrees Celsius were ideal for seedling development. At 20 and 35 °C, a similar development trend was found; however, the seeds germinated earlier at 20 °C. In addition, an overlapping temperature pattern was detected between 15 and 20 °C during the growth phase. At 10 °C, seedlings grew gradually; however, at 5 °C, growth was stable but significantly hindered, requiring additional development time. The lowered length value at 35 °C and the absence of germination at 40 °C demonstrated that temperature elevations above the ideal were deleterious to seedling development.

4.1.2. Effect of Water on seed germination and seedling growth

The summary results of growth indices and dry weight accumulation in response to two water bases, a single milliliter, and TKW percentage are presented in Tables 5 and 6. The statistical analysis based on LSD values of germinated seed numbers is shown in Tables 5 and 6. There was no significant

difference in the number of germinated seeds among different water levels for both methods. As a result, the sunflower seeds germinated successfully at all tested water levels (Tables 5 and 6).

Table 5. Mean values on germination and seedling growth traits for the various amounts of water based on 1 ml intervals.

Water (ml)	Germinated (n)	Seedling L(cm)	Seedling DW(g)
0	0.0±0.0	0.000±0.00 f	0.000±0.00 d
1	10.0±0.0	3.482±0.55 e	0.367±0.01 abc
2	10.0±0.0	4.212±1.14 e	0.372±0.02 abc
3	10.0±0.0	4.866±1.44 e	0.342±0.07 c
4	10.0±0.0	7.678±0.51 d	0.404±0.03 a
5	10.0±0.0	8.308±1.84 d	0.358±0.03 bc
6	10.0±0.0	10.468±0.94 bc	0.356±0.03 bc
7	10.0±0.0	8.118±1.45 d	0.359±0.02 bc
8	10.0±0.0	13.060±1.55 a	0.373±0.03 abc
9	10.0±0.0	14.058±0.93 a	0.363±0.02 abc
10	10.0±0.0	8.788±1.46 cd	0.377±0.04 abc
11	10.0±0.0	11.028±3.40 b	0.391±0.04 ab
12	10.0±0.0	14.538±1.42 a	0.372±0.02 abc
LSD	NS	1.906	0.041

Means are presented ±SD (n=50). Different letters denote a statistically significant difference between treatments, $p < 0.05$ following LSD (Fisher test). They began in order, with the letter (a) being the most significant. NS: Indicates that there is no significant difference between the means. L: length (cm), DW: dry weight (g), n: number.

The growth of seedlings increased significantly with water volume to the optimal level but decreased significantly as water quantity increased beyond the optimal level (Tables 5 and 6). The optimal water range for seedling length was 8,2–11,4, representing 1625–2250 percent of TKW (Table 6). Moreover, it was within the ideal range indicated by the one milliliter (8–12 ml) water-based approach (Table 5). Therefore, it can be stated that the TKW approach is more precise in optimizing germination water

requirements. The effect of different levels of water on radicles and plumules is available in the publications (HAJ SGHAIER et al., 2023).

Table 6. Mean comparison of germination and seedling growth characteristics for varying amounts of water depending on TKW%.

Water ml Of TKW	Germinated (n)	Seedling L (cm)	Seedling DW (g)
0	0.0±0.00	0.000±0.00 j	0.000±0.00 f
0.6	10.0±0.00	6.594±0.88 i	0.360±0.03 e
1.3	10.0±0.00	6.012±0.63 i	0.368±0.01 de
1.9	10.0±0.00	7.490±0.19 hi	0.384±0.01abcde
2.5	10.0±0.00	8.694±0.46 gh	0.374±0.02 bcde
3.2	10.0±0.00	8.456±0.61 gh	0.374±0.02 bcde
3.8	10.0±0.00	9.474±1.75 g	0.386±0.04 abcde
4.4	10.0±0.00	11.960±1.81 def	0.381±0.03 abcde
5	10.0±0.00	10.246±4.24 fg	0.399±0.02 ab
5.7	10.0±0.00	13.438±0.68 cd	0.405±0.01 a
6.3	10.0±0.00	12.950±0.93 cde	0.396±0.02 abc
6.9	10.0±0.00	11.550±1.31 ef	0.371±0.01 cde
7.6	10.0±0.00	13.620±0.76 bcd	0.368±0.02 de
8.2	10.0±0.00	15.726±1.46 a	0.392±0.02 abcd
8.8	10.0±0.00	13.21±0.62 cde	0.391±0.02 abcd
9.5	10.0±0.00	16.08±1.56 a	0.383±0.01 abcde
10.1	10.0±0.00	12.946±1.26 cde	0.381±0.01 abcde
10.7	10.0±0.00	14.684±1.84 abc	0.373±0.01 bcde
11.4	10.0±0.00	15.360±0.69 ab	0.378±0.02 bcde
LSD	NS	1.83	0.026

Means are presented ±SD (n=50). Different letters denote a statistically significant difference between treatments, p<0.05 following LSD (Fisher test). They began in order, with the letter (a) being the most significant. NS: Indicates that there is no significant difference between the means. L: length (cm), DW: dry weight (g), n: number.

Tables 5 and 6 display the findings of an analysis of variance performed on the dry weight of the shoot, radicle, and entire seedling. All evaluated parameters demonstrated a significant variation between the various water levels. 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 (Table 6). As a result, the seedling values' dry weight decreased at a level greater than 8.8 ml (Tables 5 and 6).

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

The effect of seed numbers on growth parameters and dry weight accumulation of seedlings is displayed in Table 7. 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 (Table 7). Therefore, seed densities of 10–12 appeared ideal for developing a sunflower crop in vitro. Radicles and plumules' response to seed density is available in the publications (HAJ SGHAIER et al., 2023).

Table 7. Collecting data on sunflower seedling response to seed density

Seed number	Seedling L (cm)	Seedling DW (g)
6	9.950±1.60	0.246±0.02 d
8	10.28±1.34	0.331±0.02 c
10	10.04±1.12	0.416±0.02 b
12	9.644 ±0.61	0.483±0.02 a
LSD	N.S	0.017

Means are presented ±SD (n=100). Different letters denote a statistically significant difference between treatments, p<0.05 following LSD (Fisher test). They began in order, with the letter (a) being

the most significant. NS: Indicates that there is no significant difference between the means. L: length (cm), DW: dry weight.

4.1.4. Effect of Antifungal methods on germination and seedling growth
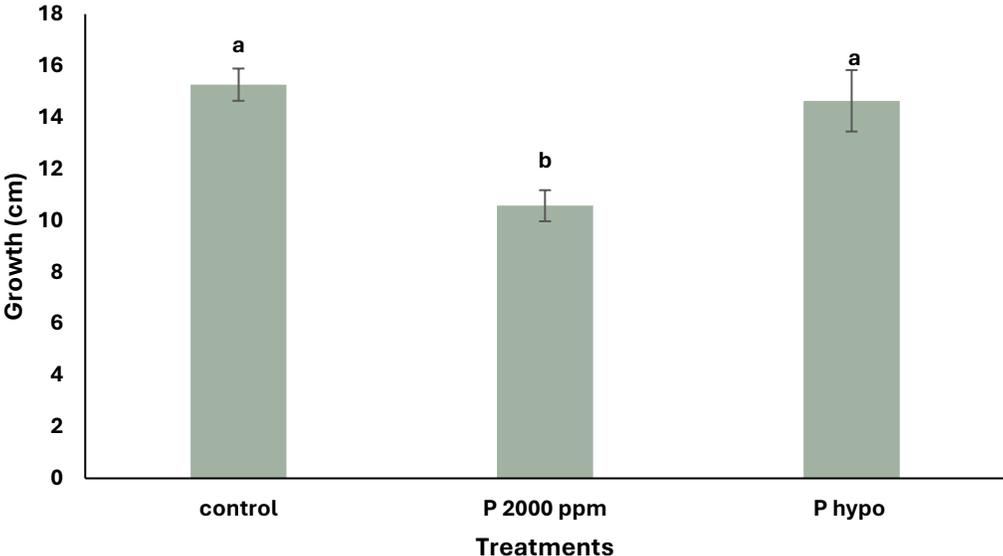
Figure 16. The growth response of the seedling to two distinct fungal seed treatment procedures. Means are presented ±SD (n=100). At p<0.05, values between treatments marked by different letters differ significantly. They began in order, with the letter (a) being the most significant.

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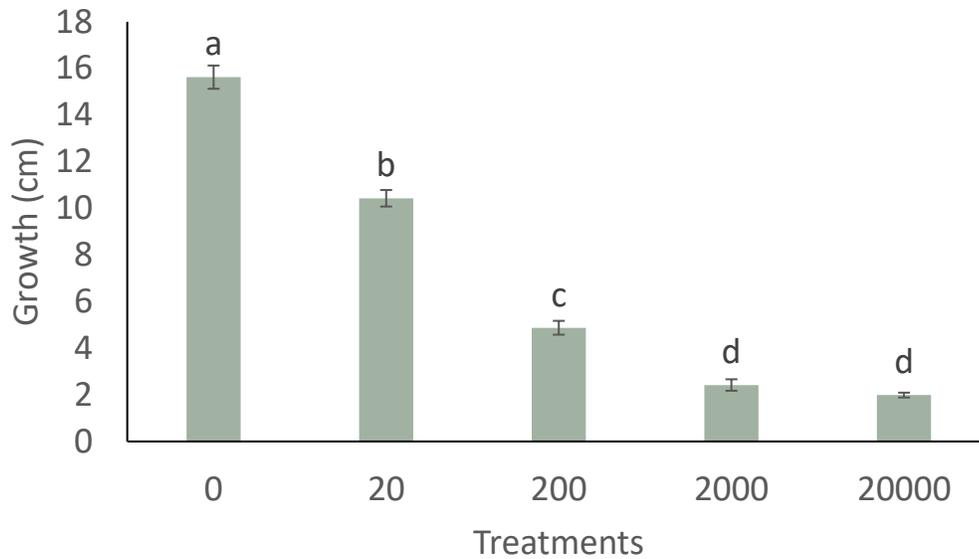
Figure 17. Growth response of seedlings to different concentrations of the fungicide Amistar Xtra.
Means are presented ±SD (n=100). At p < 0.05, values between treatments marked by different
letters differ significantly. They began in order, with the letter (a) being the most significant.

Figure 16 illustrates the recorded data and comparison results for pretreated germination seeds with antifungal Amistar Xtra and Hypo (10% Sodium hypochlorite (NaClO)). The priming of seeds with Hypo does not have a significant effect on seedling growth compared to the control, but the antifungal growth medium Amistar Xtra does suppress fungal growth of seedling growth. Consequently, its development decreased by 87% relative to the control. In addition, the findings given in Figure 17 demonstrated that, even at low doses, seedling growth dropped correspondingly as antifungal concentrations increased. Radicles and plumules' response to antifungal growth is available in the publications (HAJ SGHAIER et al., 2023).
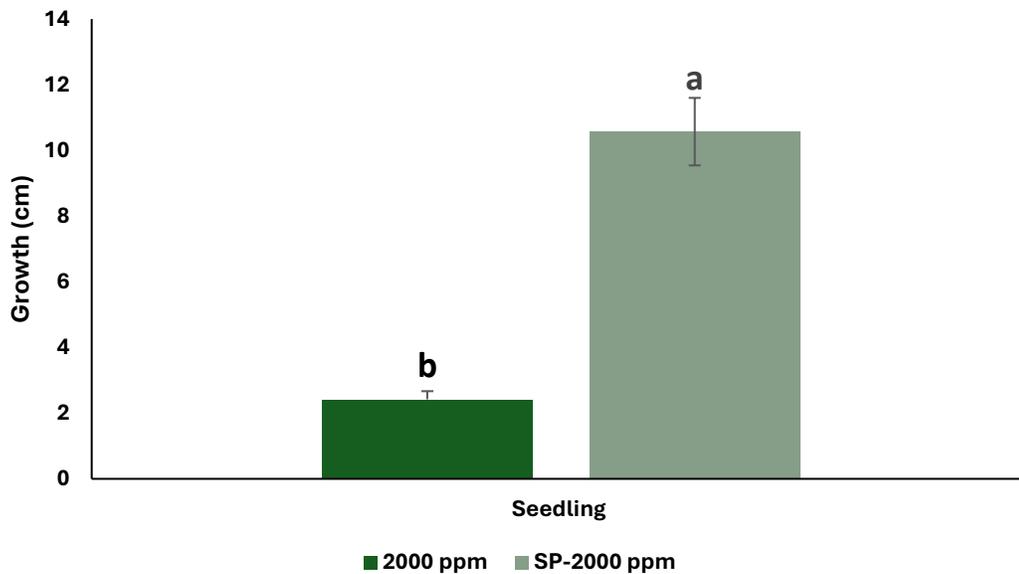
Figure 18. Responses of seedlings to two methods of fungicide control (sterilization SP-2000 ppm and growth media 2000 ppm). Means are presented ±SD (n=100). At p < 0.05, values labeled with different letters differ significantly. They began in order, with the letter (a) being the most significant.

Figure 18 contrasts the procedures of priming seeds with a fungicide solution and growing seeds in growth media containing the fungicide Amistar Xtra. Compared to the treated seeds in the growing medium, the priming approach enhanced all growth indicators significantly. Priming or pre-treating seeds can be a useful option for reducing fungal growth during in vitro seed germination.

4.2.   Field results: sunflower response to fertilizer treatment over the years

4.2.1. Effect of Year and Fertilizer Treatment on Growth and Physiological Parameters in Sunflowers

According to the two-way multivariate Anova, sunflower growth and physiology parameters are significantly different within the treatment and year factors, with the unexplained variance rate Wilk's lambda p <0,001 (Table B.5, Appendix). A higher leaf area index (LAI) is generally correlated with greater light interception and biomass accumulation, which directly influences photosynthetic capacity, water use efficiency, and overall plant productivity in sunflowers. The leaf area index (LAI) is a critical indicator of canopy development.

68
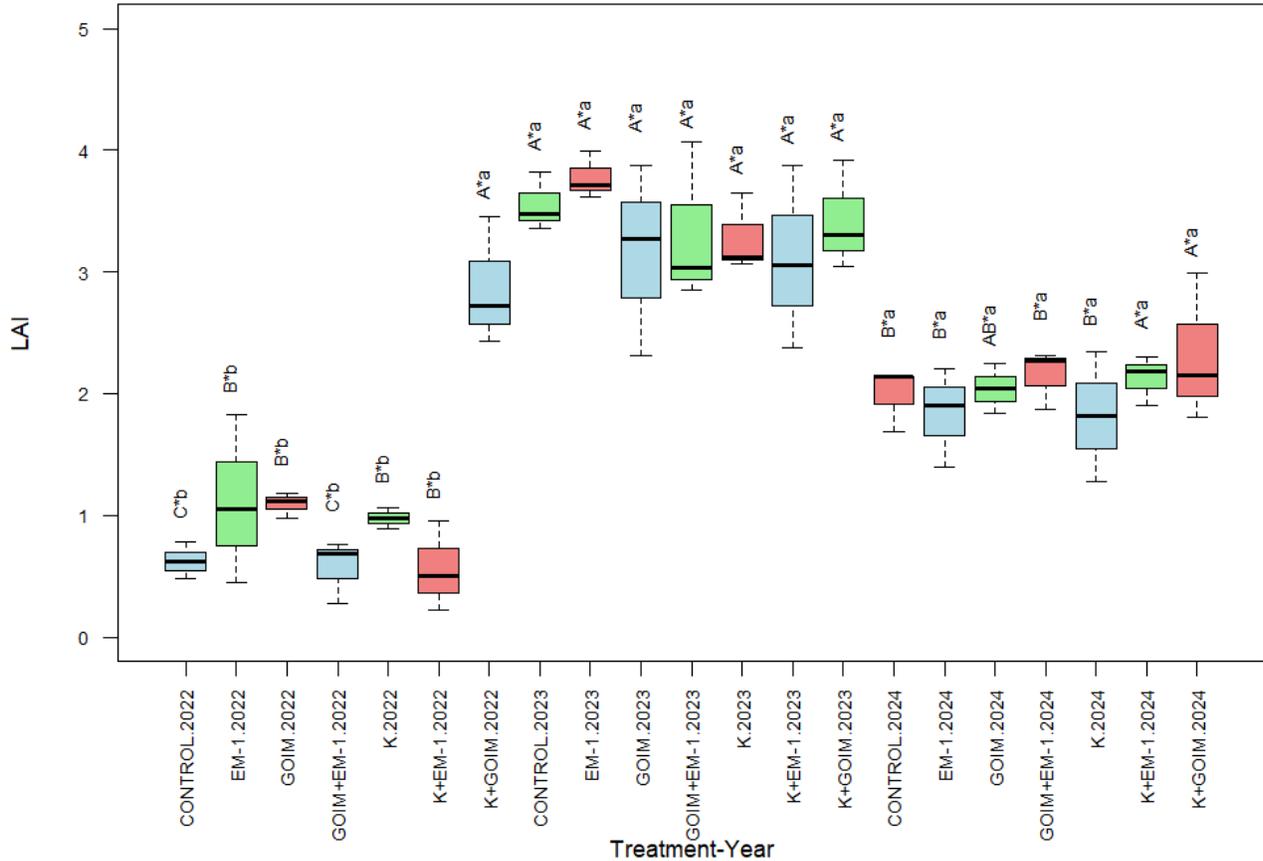
Figure 19. Effect of Treatments on Leaf Area Index LAI across Different Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).

The Leaf Area Index (LAI) was determined to have a significant year effect (F (2,42) = 139.857, p <0.001), a significant fertilizer effect (F (6,42) = 4.783, p = 0.001<0.05), and a significant year × fertilizer treatment interaction (F (2,6) = 3.377, p = 0.002<0.05) by the multivariate two-way ANOVA (Table B.5, Appendix). The LAI response of sunflower under various fertilizer treatments over a three-year period is depicted in Figure 19. The accompanying Figure illustrates the outcomes of the pairwise comparisons conducted using the Games-Howell post hoc test. All treatments were reported to have a significantly higher LAI in 2023, followed by 2024 and 2022. In 2022, the K+GOIM generated the most significant values of about 2.87. This treatment achieved the highest values in 2023 and 2024; however, it was not statistically significant. In comparison to other treatments, GOIM+EM and K+EM

exhibited the lowest values in 2022, about 0.5. The maximum LAI levels were observed in the K+GOIM treatment, as indicated by the interaction between the year 2022 and fertilizer effect.
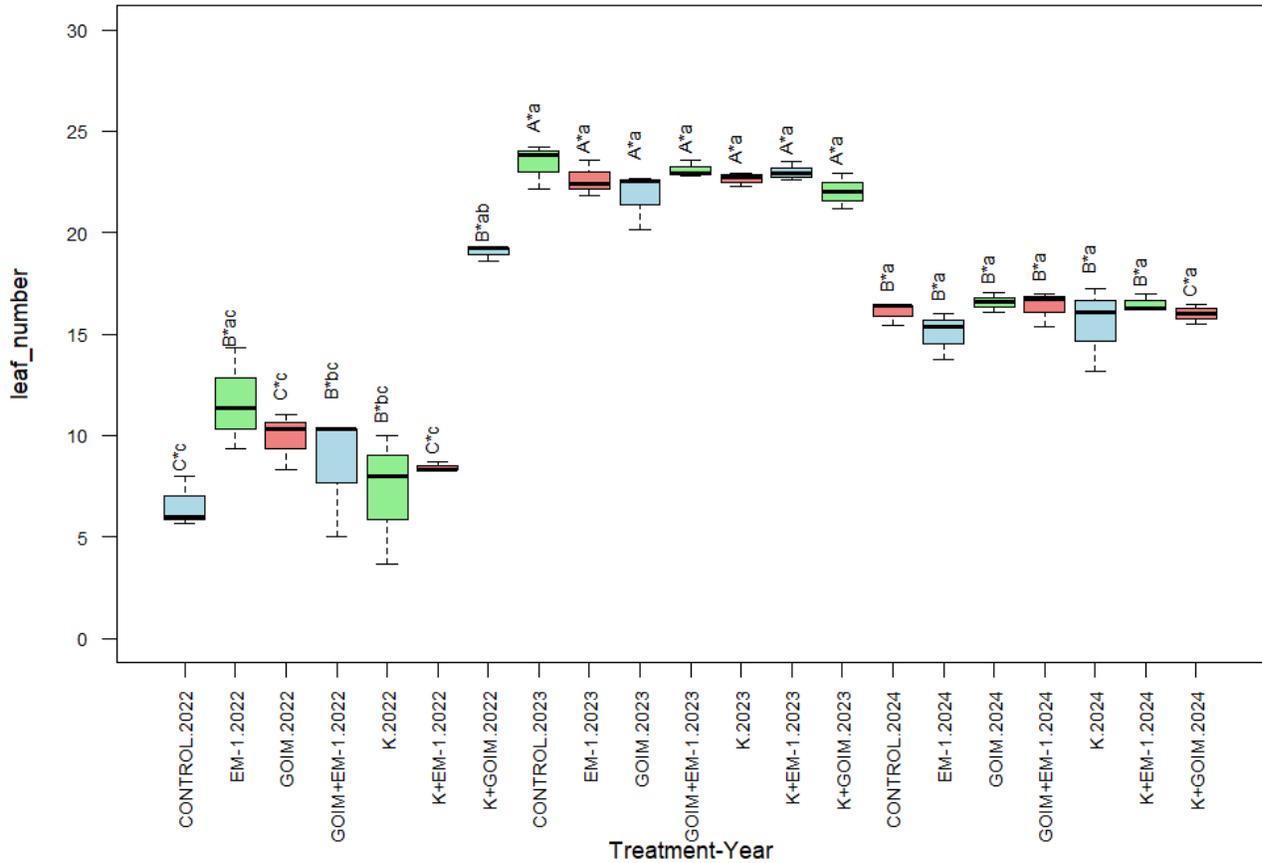
Figure 20. Effect of Treatments on number of leaves of sunflower across Different Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).

The number of leaves in sunflowers is a critical growth parameter, as it directly impacts the plant's capacity to absorb sunlight for photosynthesis, which in turn affects both biomass production and the overall yield potential. In table B.5 (Appendix), the multivariate two-way ANOVA with Bonferroni correction revealed a significant year effect ($F_{(2,42)} = 402.280$, $p<0.001$), a significant fertilizer effect ($F_{(6,42)} = 7.319$, $p<0.001$), and a significant year × fertilizer treatment interaction ($F_{(2,6)} = 10.089$, $p<0.001$) on the number of leaves. The number of leaves that a sunflower produces in response to various fertilizer treatments over three years is depicted in Figure 20. The accompanying Figure illustrates the outcomes of the pairwise comparisons conducted using the Games-Howell post hoc test.

The number of leaves was substantially higher in 2023 for all treatments, 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. The most significant fertilizers were K+GOIM and EM, as also indicated by the interaction between year and fertilizer effect (Table B.2, Appendix).
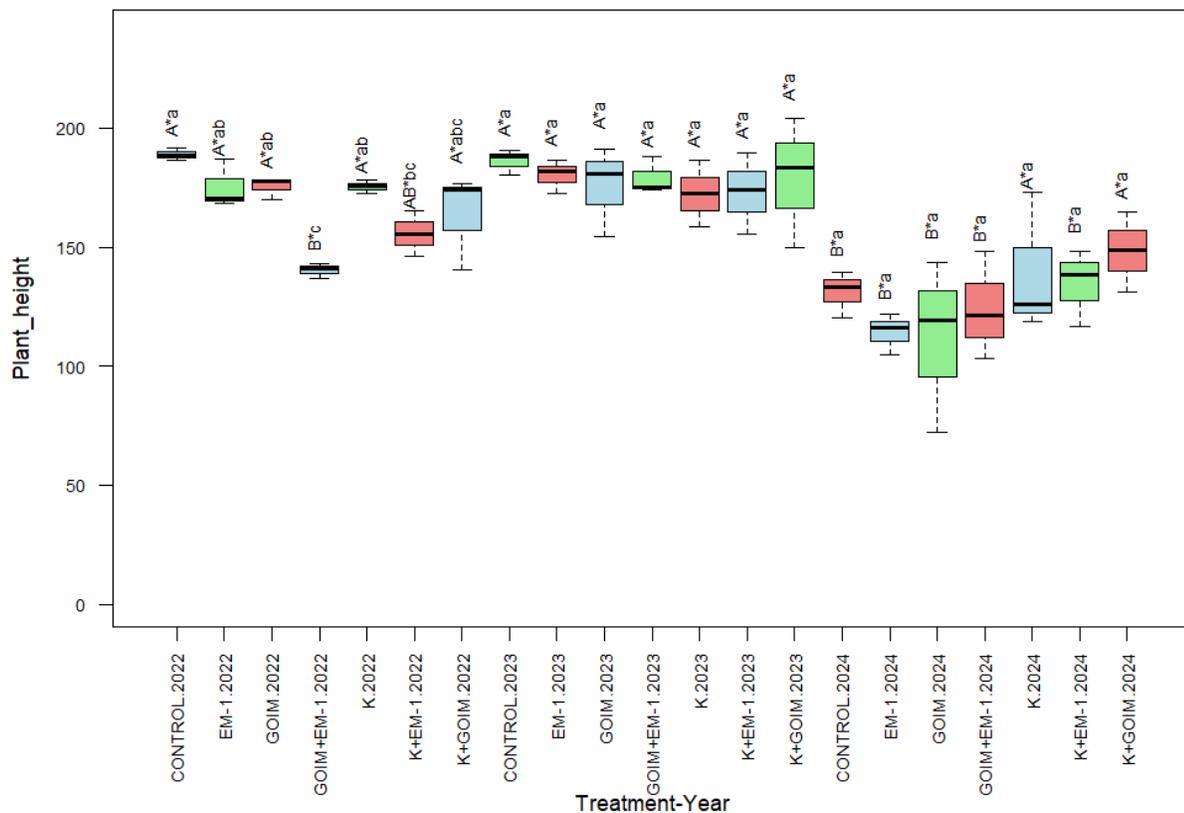
Figure 21. Effect of Treatments on Plant height of sunflower across Different Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).

Sunflowers' plant height is a critical growth metric that indicates the plant's health and vigor, frequently indicating its capacity to compete for sunlight. This can have an impact on the overall biomass and seed production of the plant. A significant year effect ($F_{(2,42)} = 50.321$, $p<0.001$) on the number of leaves was identified by the multivariate two-way ANOVA (Table B.5, Appendix). The plant height response of sunflowers to various fertilizer treatments over three years is depicted in

Figure 21. The accompanying Figure 21 illustrates the outcomes of the pairwise comparisons conducted using the Games-Howell post hoc test. The plant height was observed to be substantially higher in 2022 and 2023 for all treatments except the GOIM+EM treatment in 2022. The EM, GOIM, and K treatments resulted in substantially higher plant heights around 175 cm in 2022; however, their values do not surpass the control (189 cm). In 2024, the highest values were recorded by K+GOIM (148 cm), although the differences were not statistically significant.
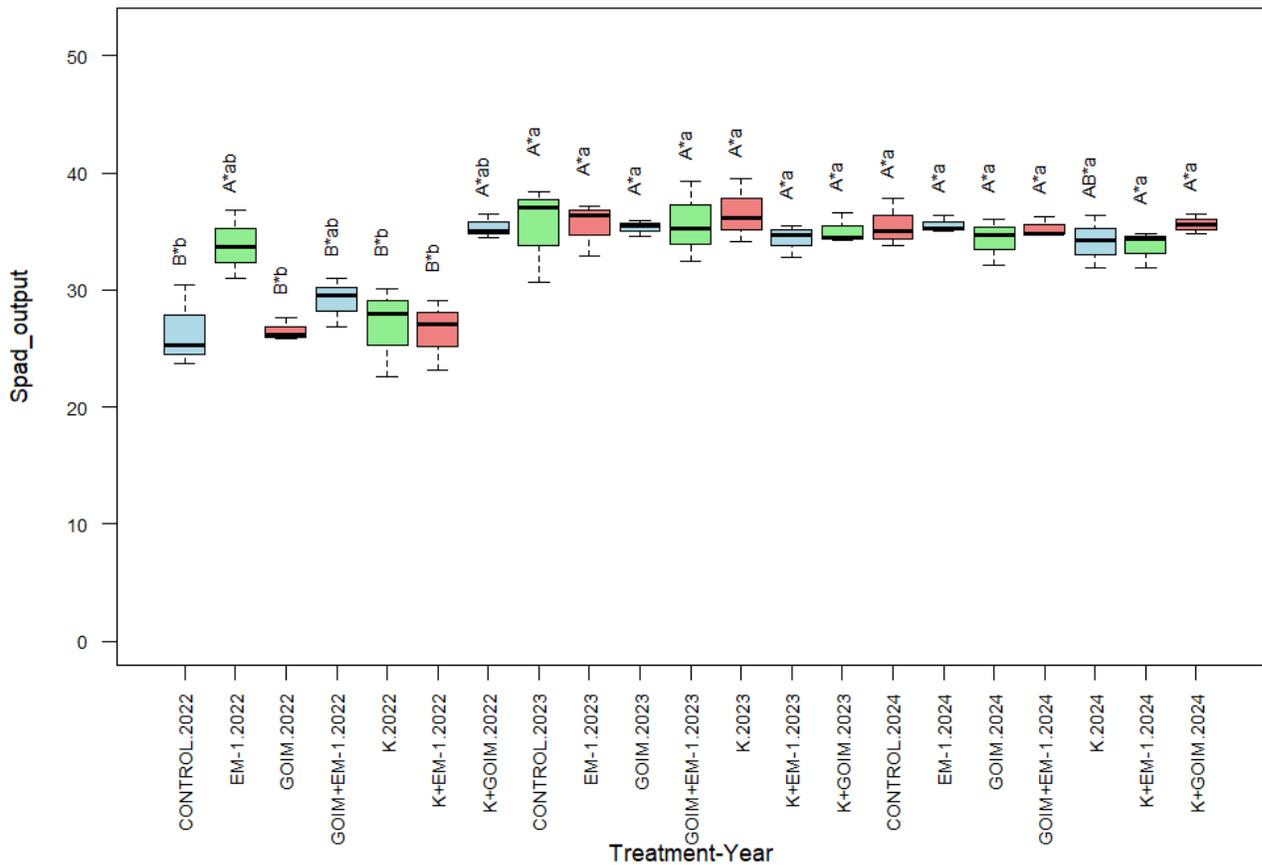
Figure 22. Effect of Treatments on SPAD output of sunflower across Different Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).

The SPAD values in sunflowers are a critical growth parameter, as they directly impact on the plant's capacity to absorb sunlight for photosynthesis, which in turn affects both biomass production and the overall yield potential. In the table B.5 (Appendix), the multivariate two-way ANOVA revealed a significant year effect (F $(2,42)$ = 44.921, $p < 0.001$), a significant fertilizer effect (F $(6,42)$ = 3.553,

p < 0.001), and a significant year × fertilizer treatment interaction (F (2,6) = 2.476, p = 0.015 < 0.05) on SPAD output. Figure 22 illustrates the impact of fertilizer treatment and year on the SPAD of sunflowers treated with various fertilizers over three years. The accompanying Figure 22 illustrates the outcomes of the pairwise comparisons conducted using the Games-Howell post hoc test. The output values of SPAD are reported to be substantially higher in 2023 and 2024 for all treatments. In 2022, K+GOIM and EM generated the most significant values, 35.3 and 33.8, respectively. The SPAD values for the control plants were registered at 26.46. For the other years, no substantial disparity was identified. The most significant treatments were K+GOIM and EM, as indicated by the interaction between year and fertilizer effect. In summary, the SPAD values and leaf number were increased by K+GOIM and EM. The Leaf Area Index was significantly enhanced by K+GOIM, GOIM+EM, and GOIM. Furthermore, the GOIM, K, and K+GOIM treatments all increased stem diameter.

4.2.2. Effect of fertilizers treatment and year on Sunflower yield attributes

The two-way multivariate ANOVA indicates that the sunflower yield parameters are substantially different within the treatment and year factors, with an unexplained variance rate of Wilk's lambda p<0.001 (Table B.6, Appendix).
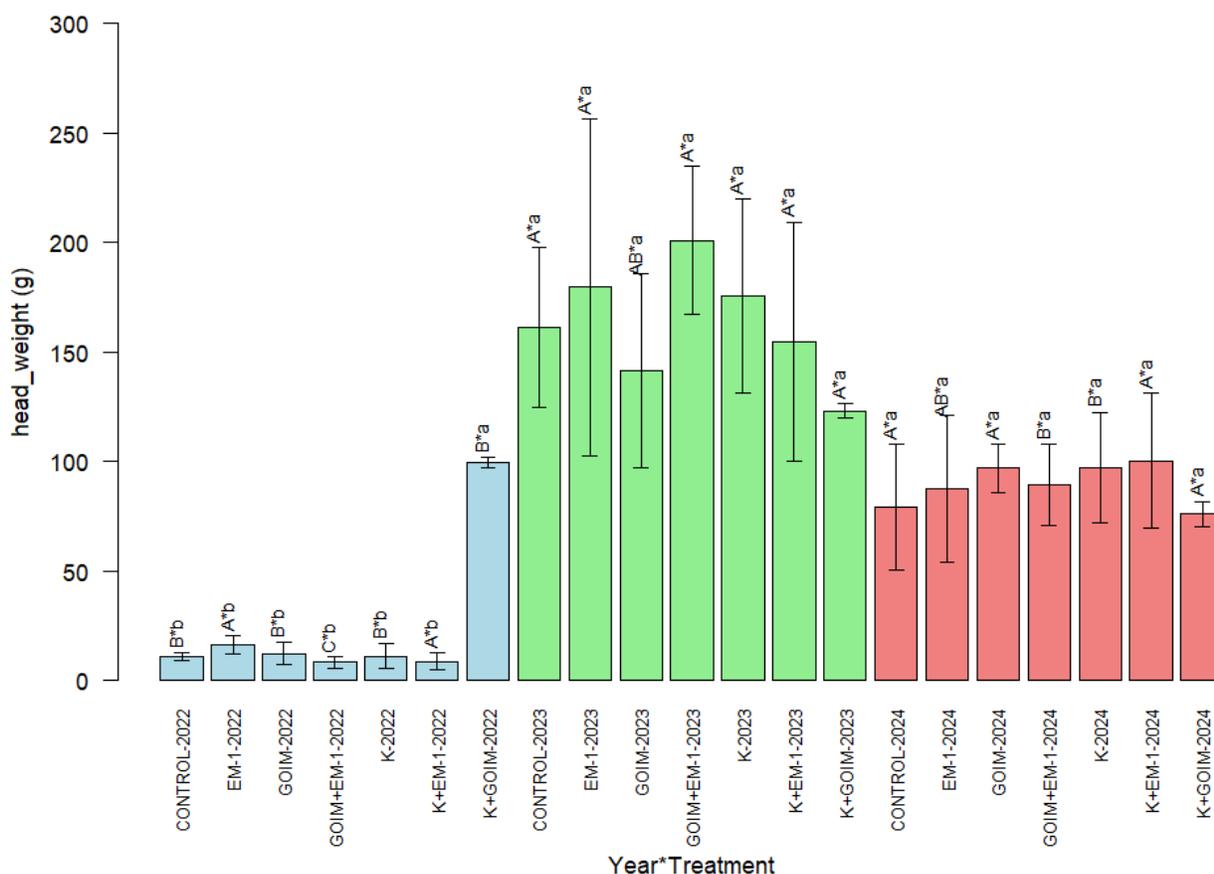
Figure 23. Sunflower Head Weight Response to Fertilization Treatments Over Three Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).

The sunflower head's weight is a reliable metric for evaluating seed production, which in turn affects oil yield, as it takes into account the bulk of seeds. The influence of years and fertilization application on the weight of sunflower heads over three growing seasons is depicted in Figure 23. The subsequent multivariate two-way ANOVA analyses revealed a significant treatment effect $F_{(6,42)} = 2.807$, $p = 0.007 < 0.05$, and a significant interaction effect between year and treatment $F_{(2,6)} = 108.786$, $p < 0.001$, on head weight (Table B.6, Appendix). The adjoining Figure 23 illustrates the outcomes of the post hoc pairwise comparison, which were obtained through the application of Games-Howell's method. Head weight is substantially determined by the year variable. 2023 was the year with the highest head weight (200g), with 2024 and 2022 following closely behind. Although the multivariate ANOVA did not disclose a significant treatment effect on head weight (Table 14, Appendix), the post

74

hoc analysis revealed significant treatment differences. These differences were attributed to the specific pairwise differences. The sunflowers' head weight was significantly increased as a consequence of the combination of potassium, organic nitrogen, and inorganic nitrogen (K+GOIM) in 2022, about 99.5 g. The head weight of the K+GOIM treatment was higher in 2023 (122 g) than in 2022, but it did not surpass the head weight of certain other treatments. Compared to the control, the K+GOIM remained elevated in 2024, but it was lower than the levels recorded in 2023. The greatest head weights were recorded by GOIM+EM (200.85 g), EM (179.5g), and K (175 g) in 2023, and K+EM (100g) and GOIM (97g) in 2024. Nevertheless, these findings were not statistically significant in either of the two years.
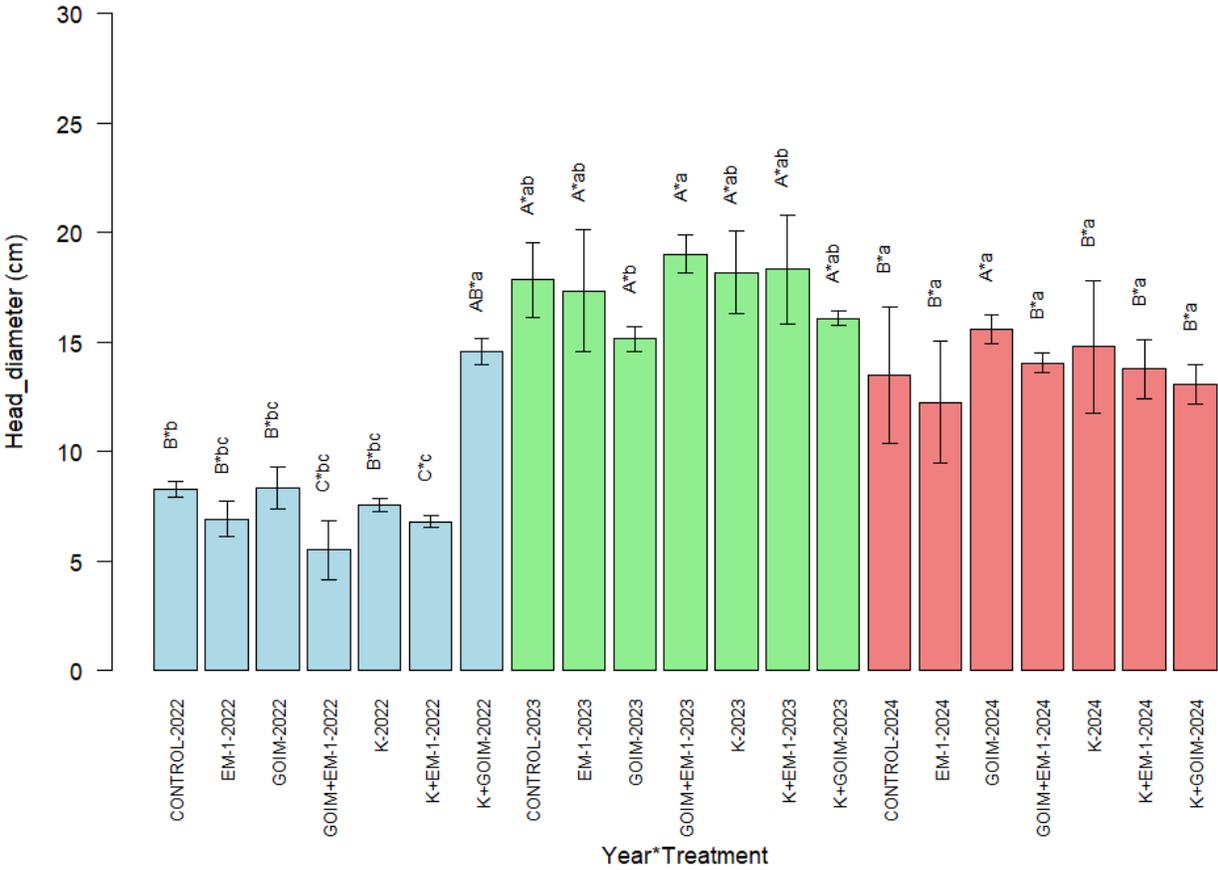
*Figure 24. Sunflower Head diameter Response to Fertilization Treatments Over Three Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).*

The plant's seed-forming capacity is indicated by the diameter of the head, with larger heads typically generating a greater number of seeds, resulting in higher seed and oil yields. The impact of fertilization treatments and the year on the diameter of the sunflower head throughout three growing seasons is depicted in Figure 24. The multivariate two-way ANOVA revealed a significant main effect of year ($F_{(2,42)} = 167.683$, $p < 0.001$) and a significant interaction between fertilizer treatment and year ($F_{(2,6)} = 5.668$, $p < 0.001$) on head diameter (Table B.6, Appendix). Figure 24 illustrates pairwise comparisons from the Games-Howell post hoc test. The head diameter was significantly influenced by the year. In comparison to 2022, the head diameter considerably increased in 2023 by approximately 50%. The treatment effect was significant in 2022 and 2023. The head diameter of sunflowers considerably increased in 2022 and 2023 as a result of the combination of potassium, organic nitrogen, and inorganic nitrogen (K+GOIM), 14.56 cm. In 2024, this treatment continued to generate a greater value than certain other treatments. The head diameter values of sunflowers treated with K+GOIM were substantially higher in 2023 and 2022 than in 2024. The post-hoc results also indicated a substantial difference in treatment for the head diameter, even though the aggregate multivariate analysis did not reveal a significant effect. The treatments GOIM + EM produced substantially higher values in 2023 (19 cm) than all other treatments, followed by K + EM (18 cm) and K (18 cm). The head diameter values of sunflowers treated with GOIM+EM were substantially higher in 2023, followed by 2024 and 2022. The K and GOIM produced the highest head diameter in 2024, 14.6 and 15 cm, respectively. Nevertheless, these findings were not statistically significant in either of the two years (Table B.1, Appendix).
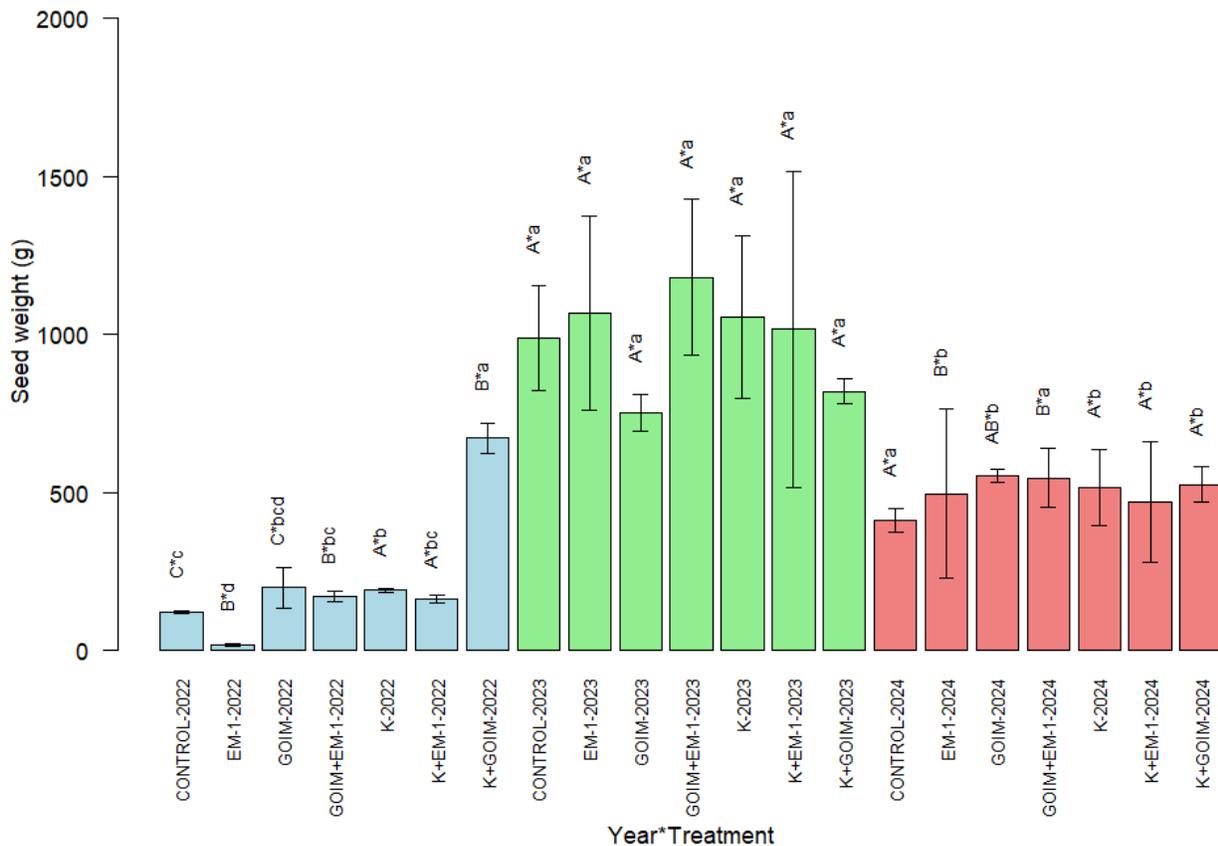
Figure 25. Sunflower seed weight Response to Fertilization Treatments Over Three Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).

The sunflower seed weight response to various fertilization regimens from 2022 to 2024 is illustrated in Figure 25. A significant effect of fertilization treatment (F (6,42) = 101.4, p < 0.001) and a significant interaction between year and treatment (F (2,6) = 2.688, p = 0.009) were observed in the multivariate two-way ANOVA model (Table B.6, Appendix). The weight of seeds has increased significantly from 2022 to 2024. The most significant value was documented in 2023, with 2022 and 2024 following closely behind. The treatment effect was particularly pronounced in 2022 and 2024, as indicated by the post hoc analysis. The treatment K+GOIM achieved the most significant seed weight values in 2022 (672 g). In comparison to 2022, the seed weight values of sunflowers treated with K+GOIM were substantially higher in 2023 (820 g) and 2024 (524 g). The treatment GOIM+EM registered the maximum seed weight of sunflower among all fertilizer treatments in 2024 (1180 g). In

2023, the seed weight of sunflowers treated with GOIM+EM was significantly higher than that of 2022 (171 g) and 2024 (546 g) (Table B.1, Appendix).
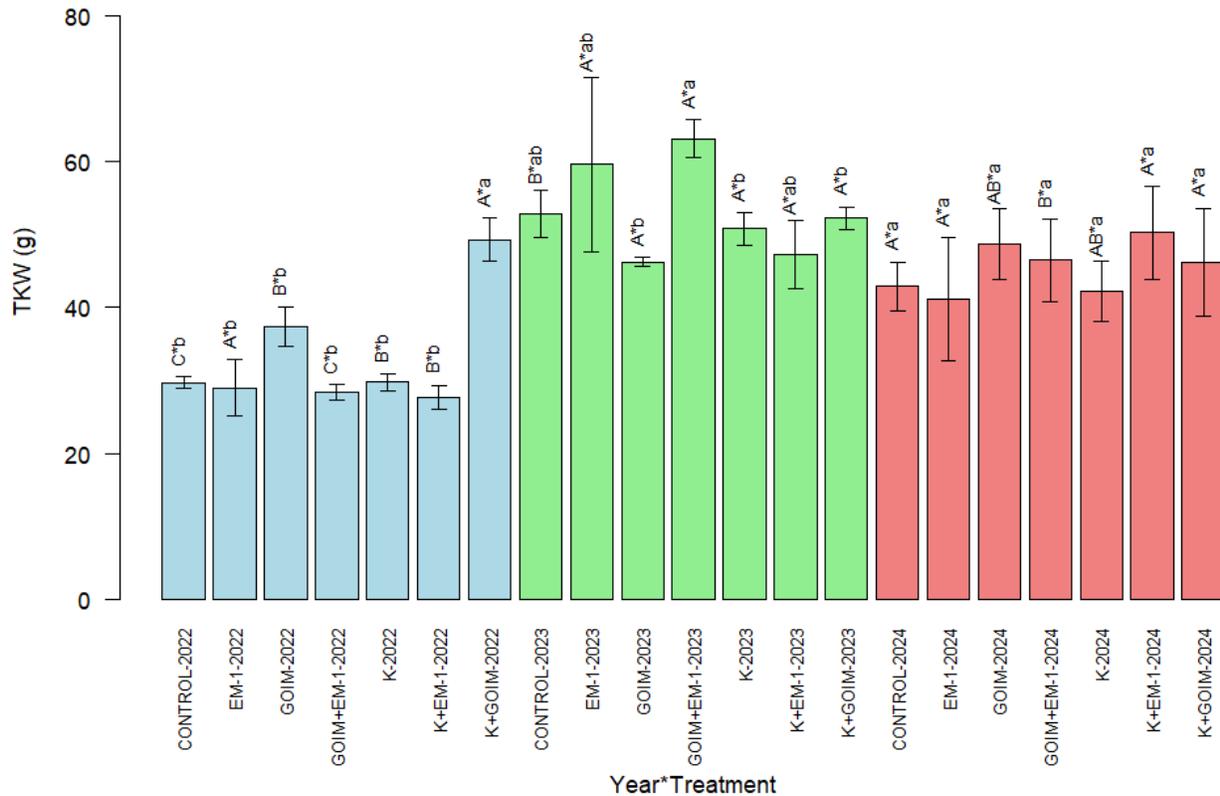
*Figure 26. Sunflower TKW Response to Fertilization Treatments Over Three Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).*

A significant year effect ($F_{(2,42)}$ =95.523, p<0.001), treatment effect ($F_{(6,42)}$ =3.396, p=0.008), and significant interaction effect of year and fertilizer treatment ($F_{(2,6)}$ =5.703, p<0.001) on the TKW were disclosed by the follow-up multivariate two-way multivariate ANOVA tests (Table B.6, Appendix). Figure 26 illustrates the post-hoc test results obtained through pairwise comparison using Games-Howell's method. The response of TKW to sunflower fertilization from 2022 to 2024 is depicted in Figure 26. In the years 2022 and 2023, the impact of fertilizer treatment was significant. The treatment K+GOIM achieved the highest TKW values in 2022 (49.25 g). In comparison to 2022, this treatment resulted in the maximum TKW in 2023 (52 g) and 2024 (46 g). In 2023, the most

significant values of TKW were achieved by the treatments GOIM + EM 2023 (63g), followed by EM (59.58g) and K + EM (50.2g). In 2024, those fertilizers achieved the highest values. TKW experienced a substantial increase in all treatments from 2022 to 2023, with the most notable increases in GOIM+EM (55%), K (37.6%), and K+EM (842.32%) (Table B.1, Appendix).

The result of the subsequent multivariate two-way ANOVA tests of yield was a significant year effect, $F_{(2,42)} = 71.064$, $p < 0.001$, and a significant interaction effect of year and fertilizer treatment, $F_{(2,6)} = 2.539$, $p = 0.013 < 0.05$ (Table B.6, Appendix) on the yield. The K+GOIM treatment yielded the highest sunflower yield in 2022 (2.96 t/ha) and 2024 (2.45 t/ha), as evidenced by Table B.3 (Appendix). The K and GOIM treatments followed. Compared to other treatments, the yield of sunflowers was higher in 2023 for K (4.023 t/ha) and EM (4.6 t/ha), but no effect was detected.

The results of this investigation indicate that sunflower yield parameters, such as head weight, head diameter, seed weight, and TKW, varied substantially across various fertilizer regimens and years. Throughout the study period, the regimens that combined potassium, organic nitrogen, and inorganic nitrogen (K+GOIM) consistently yielded substantially higher values for head weight, head diameter, TKW, seed weight, and yield, with a particular emphasis on 2022. Furthermore, treatments that combined organic nitrogen and inorganic nitrogen and effective microorganism (GOIM+EM) also resulted in increased yield parameters, particularly in 2023. Although the yield parameters of GOIM were higher in 2024, these differences were not consistently significant when compared to the results of previous years. In 2023, the head diameter was enhanced by the K+EM treatment, while in 2024, the head weight and TKW were enhanced. The treatment K enhanced the number of seeds per head in 2022, the head diameter and seed number per head in 2023, and the yield, head weight, and seed weight in 2024. In general, the findings indicate that the combination of organic and inorganic fertilizers, particularly remedies such as K+GOIM and GOIM+EM, can enhance sunflower yield parameters, although the extent of this improvement may differ depending on the year.

4.2.3. Effect of fertilizers treatment and year on oil content, moisture content, and protein content

The two-way multivariate ANOVA indicates that the sunflower seed quality parameters are substantially different within the treatment and year factors, with an unexplained variance rate of Wilk's lambda $p < 0.001$ (Table B.7, Appendix). Oil content in sunflower seeds is a critical parameter that directly impacts quality and yield. The multivariate two-way ANOVA demonstrated a significant

main effect of year (F (2,42) = 33.521, p < 0.001) and a significant interaction between year and fertilizer treatment (F (2,6) = 6.532, p < 0.001) on oil content.
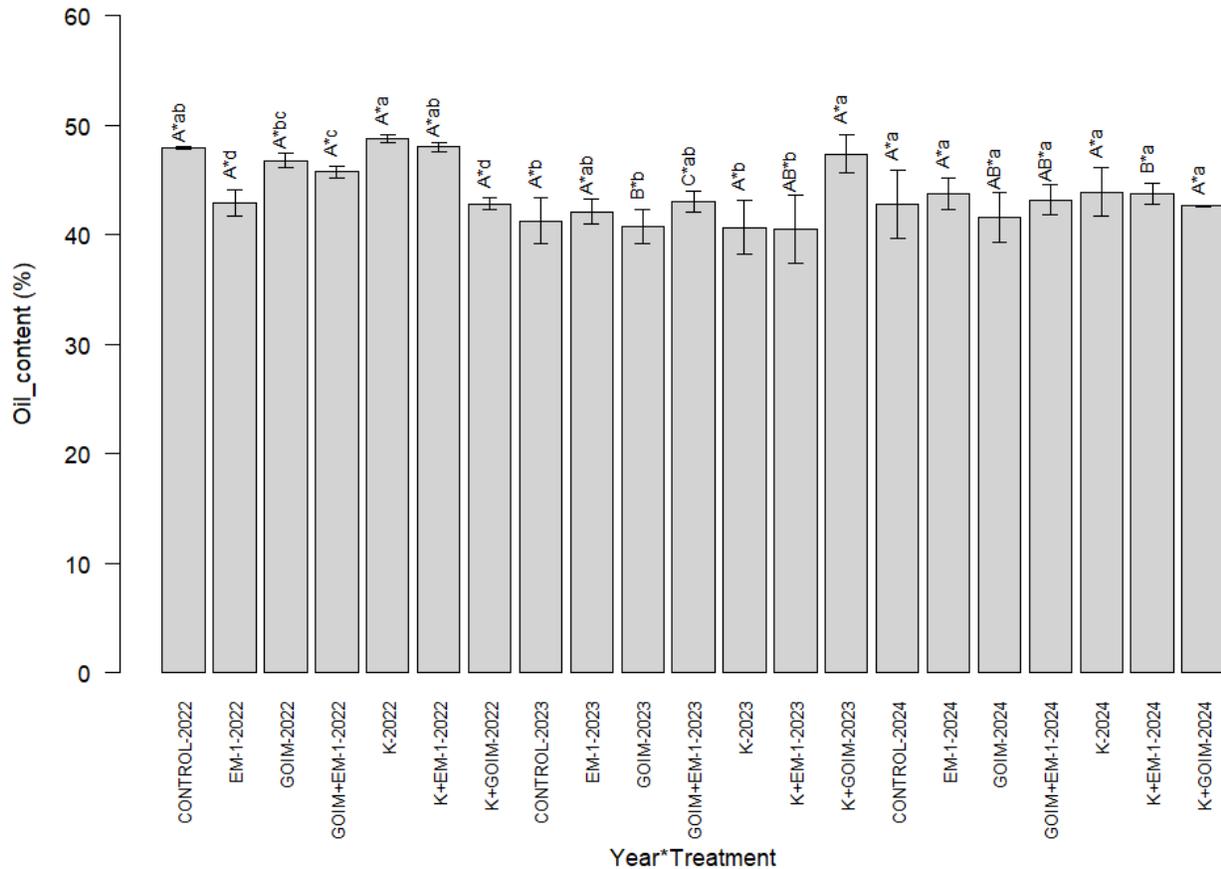
*Figure 27. Effect of Treatments on oil content Across Different Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).*

Table B.7 (Appendix) presents pairwise comparisons that were performed using the Games-Howell post hoc test. There was a substantial year-effect observed; the oil content was substantially increased in 2022. In 2023, the year effect was observed for certain fertilizers, including GOIM and GOIM+EM, and in 2024 for K+EM. K and K+EM were the most significant treatments in 2022, producing the maximum level of oil content (48%) in comparison to other treatments, as indicated by the interaction between year and fertilizer treatment (Figure 27). K+GOIM generated the maximum oil content in 2023 (48%), with GOIM+EM following in this order (43%) (Table B1, Appendix).
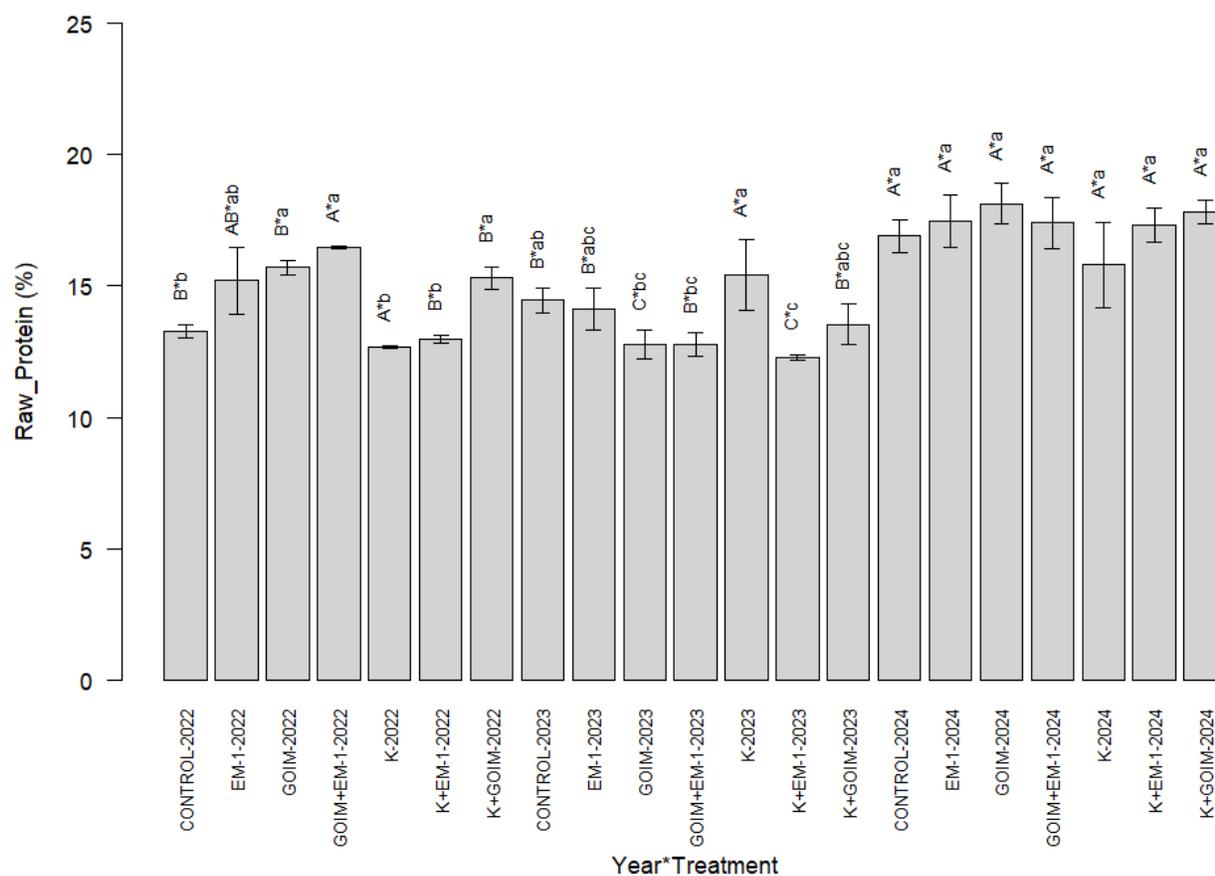
Figure 28. Effect of Treatments on protein Across Different Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).

A significant main effect of year ($F_{(2,42)} = 134.074$, $p<0.001$), a significant effect of fertilizer treatment ($F_{(6,42)} = 5.174$, $p<0.001$), and a significant interaction between year and fertilizer treatment ($F_{(2,6)} = 8.273$, $p<0.001$) on protein levels were determined by the multivariate two-way ANOVA with. Using the Games-Howell post hoc test, pairwise comparisons are illustrated in Table B.7 (Appendix). The nutritional value of sunflower seeds is significantly influenced by their raw protein content. The unprocessed protein response to fertilizer treatment and year factors is emphasized in Figure 28. From 2022 to 2023, there was a substantial decrease in the protein of GOIM, EM, GOIM+EM, and K+EM. The remaining treatments did not exhibit any significant differences. The protein levels during 2022 were highest in treatments GOIM (15.7%), GOIM+EM (16.46%), and K+GOIM (15.33%). The protein levels were significantly lower in the treatments K and K+EM, as well as the control. In 2023, the K treatment exhibited the most significant outcome (15.43%),

81

resulting in the highest protein content among the other treatments. In 2024, it appears that multiple treatments (K, K+EM, and GOIM+EM) have similarly high protein content, with no distinct singular treatment being the most dominant. The most significant treatment in 2022 was GOIM+EM, while in 2023, K was the most significant treatment, as indicated by the interaction between year and fertilizer treatment. Both K and GOIM+EM were equally significant in achieving the maximum protein content in 2024 (Table B1, Appendix).
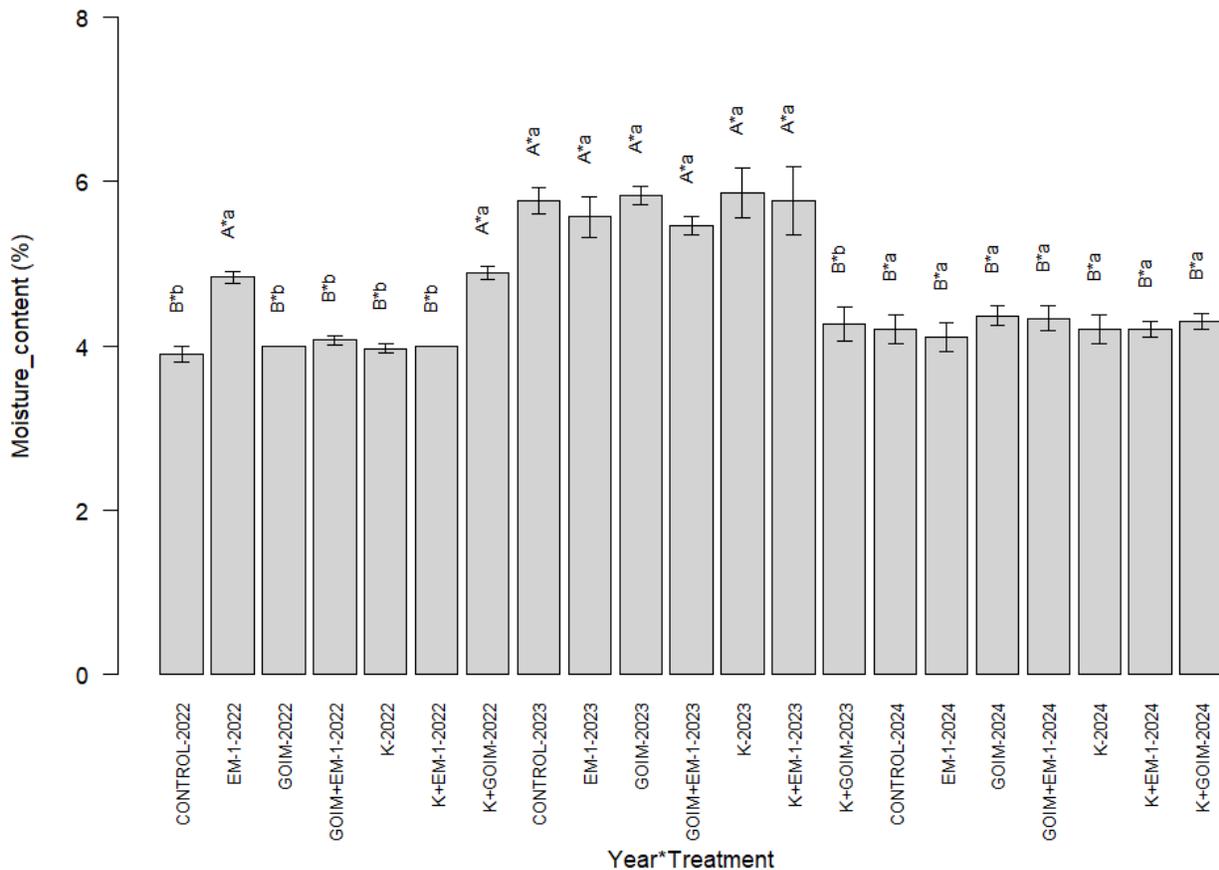
*Figure 29. Effect of Treatments on moisture content Across Different Years. Means are presented ± SD (n=3). Different letters denote a statistically significant Year Effects (Capital Letters) and Treatment Effects (Small Letters). They began in order with letter (a/A) being the most significant. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment).*

In terms of moisture content, the multivariate two-way ANOVA revealed a significant year effect ($F_{(2,42)} = 393.453$, $p < 0.001$), a significant fertilizer effect ($F_{(6,42)} = 3.675$, $p = 0.005$), and a significant year and fertilizer treatment interaction ($F_{(2,6)} = 25.085$, $p < 0.001$) (Table B.7, Appendix). The accompanying Figure 29 illustrates the outcomes of the pairwise comparisons conducted using the

Games-Howell post hoc test. The moisture content offered significant insights into the water dynamics within the crop, enabling the evaluation of its physiological response and overall performance under fertilizer treatment throughout the year. The moisture content response of sunflowers to various fertilizer treatments over a three-year period is depicted in Figure 29. The moisture content of all treatments was reported to be significantly higher in 2023. There was no discernible distinction between 2022 and 2024, with the exception of the treatments EM and K+GOIM, which exhibited a substantially higher level of efficacy in 2022 than in 2024. In 2022, the moisture content of EM (4.83%) and K+GOIM (4.88%) was the highest, as indicated by the interaction between year and fertilizer effects. Their moisture content values were higher in 2023 and 2024; however, no substantial differences were identified (Table B1, Appendix). In summary, the moisture content of sunflower seeds was enhanced by the K+GOIM and EM fertilizers. In 2022, the protein content was improved by GOIM, GOIM+EM, and K+GOIM treatments. However, in 2023, the protein content was improved by K. The oil content was improved in 2022 by the treatments K+EM and K, and in 2023, the oil content was improved by K+GOIM and GOIM+EM.

4.3.   Effect of Year and fertilizers treatment on fatty acid composition

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 (Wilks' lambda is significant $p<0.01$) (Table B.8, Appendix).

4.3.1. Monounsaturated fatty acids

Oleic acid (C18:1) is a monounsaturated fatty acid that has a substantial impact on the quality of sunflower oil. It is present in sunflower oil at an average concentration of 57-86%. The oleic acid level was significantly influenced by the treatment $F_{(6,42)} = 9.442$, $p<0.001$, the year $F_{(2,42)} = 2611.38$, $p<0.001$, and the interaction effect of the year and fertilizer treatment $F_{(6,12)} = 15.043$, $p<0.001$ (Table B.8, Appendix). The results of the multivariate two-way ANOVA and pairwise comparison post hoc tests, which were derived using Games-Howell's method, are presented in Table 4 (Appendix). In 2022 and 2024, the oleic acid values were higher by 70% and 84%, respectively. Significant fertilization treatments were identified in 2022 and 2024. In 2022, the treatments GOIM+EM

(79.65%) and K (76.25%) produced the highest concentrations of oleic acid. In comparison to other treatments, those treatments obtained higher values in 2023. In 2024, the oil from the K+GOIM, GOIM+ EM, and EM treatments demonstrated the highest value of oleic acid (86%) in comparison to the other treatments; however, no significant difference was detected for this year. In sunflower oil, eicosenoic acid (C20:1) is another monounsaturated fatty acid that is present in a lower percentage than oleic acid (0.14-0.28%). The concentration of eicosanoic acid fluctuated between 2022 and 2024. This acid exhibited a substantial year effect ($F_{(2,42)} = 79.345$, $p < 0.001$ (Table B.8, Appendix). The level of eicosenoic increased for all treatments from 2022 to 2024, except the control and the K+EM treatment. This increase was approximately 17%. This fatty acid exhibited a significant interaction effect between year and treatment ($F_{(2,6)} = 2.067$, $p=0.041<0.05$). In comparison to the other treatments, the oil from treatment K exhibited the highest eicosenoic value (0.28%) (Table B.4, Appendix). In conclusion, the K treatment notably increased the levels of both eicosanoid and oleic acids, the key monounsaturated fatty acids. Among the treatments, GOIM+EM produced the highest oleic acid content, followed in descending order by the K+EM and GOIM treatments.

4.3.2. Saturated fatty acid

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. Additionally, the C16:0 concentration was examined. It is present in sunflower oil at an average concentration of 3.69-5.33%. Palmitic acid was significantly influenced by the year in the follow-up multivariate two-way ANOVA tests ($F_{(6,42)} = 151.130$, $p<0.001$) (Table B.8, Appendix). Table B.4 (Appendix) displays the results of the ANOVA and pairwise comparison post hoc tests obtained through the use of Games-Howell's method. In 2023, this acid was determined to be highly significant for all treatments, with a 19% increase. The multivariate two-way ANOVA tests on the stearic acid (C18:0) in the table revealed a significant year effect $F_{(2,42)} = 115.220$, $p<0.001$, and a significant interaction effect of year and fertilizers treatment $F_{(2,6)} = 2.699$, $p= 0.009 < 0.05$ (Table B.8, Appendix). Table 4 (Appendix) displays the post-hoc test results obtained through pairwise comparison using Games-Howell's method. The concentration of stearic acid fluctuated over the years from 2.71% to 4.32%; 2023 was the year in which it increased the most, followed by 2022 and 2024. In 2023, the oils from the K+GOIM treatment exhibited a 47% increase, while K experienced a 37% increase. There was no discernible distinction between 2022 and 2024. In 2022, the statistical analysis revealed a significant interaction between fertilizer treatment and year. In comparison to other treatments, the treatment K+GOIM recorded the highest values of

stearic acid percentage (3.28%), followed by EM (3.22%), GOIM (2.94%), and GOIM+EM (3.07%). The oil from K+GOIM (4.03%), K (4.32%), and GOIM (4.03%) produced the highest values of stearic acid in 2023 without significant difference. And in 2024, the treatment GOIM (3%), and K+EM (2.84%) increased the stearic acid in the oil without any one treatment standing out as significantly better. 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. In the two-way multivariate ANOVA tests on the lignoceric acid (C24:0), the statistical analysis revealed a significant year effect $F_{(2,42)} = 324.759$, $p < 0.001$, a treatment effect $F_{(6,42)} = 2.534$, $P = 0.035 < 0.05$, and a significant interaction effect of year and fertilizer treatment $F_{(2,6)} = 3.718$, $P = 0.001 < 0.05$ (Table B.8, Appendix). Table B.4 (Appendix) displays the post-hoc test results obtained through pairwise comparison using Games-Howell's method. 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 (Table B.4, Appendix).

In 2023, a substantial increase in myristic acid (C14:0), present at 0.2-0.43 % in sunflower oil was observed in all treatments ($F_{(2,42)} = 79.143$, $p < 0.001$) (Table B.8, Appendix), 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% (Table B.4, Appendix). Margaric acid (C17:0), a saturated fatty acid, exhibited a significant effect of year ($F_{(2,42)} = 33.693$, $p < 0.001$) (Table B.8, Appendix). The oil from GOIM, K+EM, and K+GOIM treatments were the only ones to exhibit this effect. In 2023, the oil from these treatments exhibited substantially higher Margaric acid values with 23% higher than those of 2022 and 2024 (Table B.4, Appendix). The arachidic acid (C20:0) exhibited a significant year effect ($F_{(2,42)} = 33,116$, $p < 0.001$) for all treatments, except the oil from K and K+EM treatments, which were not significant in any of the years (Table B.8, Appendix). The arachidic acid level in 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 years observed. 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 (F (2,6) =2.095, p=0.039<0.05) (Table B.8, Appendix). 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 arachidic acid in 2024, and no single treatment was significantly superior to the others (Table B.4, Appendix). Tricosanoic acid C23:0 exhibited a significant year effect (F (2,42) =65,573, p<0.001). 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 (F (2,6) =3.25 p=0.015 <0.05) was significantly higher (Table B.8, Appendix). The year had a significant effect on the Behenic acid C22:0 acid (F (2,42) = 120.923, p < 0.001) (Table B.8, Appendix). The oils from the 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 (Table B.8, Appendix). In summary, the treatment GOIM resulted in an increase in the concentration of stearic acid, arachidic and lignoceric acid, behenic acid, and tricosanoic acid. Stearic acid, a critical acid in saturated fatty acids, was elevated by both treatments, K+GOIM and EM. The lignoceric and arachidic, stearic, behenic, and tricosanoic acids were increased by both GOIM+EM and K.

4.3.3. 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%. The linoleic acid was significantly influenced by the year (F (2,42) =3872, p<0.001) and treatments (F (6,42) =13.282, p<0.001) in the follow-up multivariate two-way ANOVA tests. Additionally, there was a significant interaction effect between the year and fertilizer treatment (F (2,6) =18.772, p<0.001) (Table 15, Appendix). Table B.4, Appendix presents the statistical effect results of fertilization treatment and year of linoleic acid from 2022 to 2024, as determined by post hoc (Games Howell). 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 (F (2,42) =304.6, p<0.001) for all treatments, followed by 2022 and 2024 (Table B.8, Appendix). The table displays the post-hoc outcome of the multivariate ANOVA for α-

linolenic acid. 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 (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.

4.3.4. Effect of fertilizers treatment and year on stability of oil

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, 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 subsequent multivariate two-way ANOVA tests of mono-unsaturated MUFA demonstrated a significant year effect $F_{(2,42)} = 4046.41$, $p < 0.001$, a treatment effect $F_{(6,42)} = 13.662$, $p < 0.001$, and a significant interaction effect of year and fertilizer treatment $F_{(2,6)} = 19.330$, $p < 0.001$ (Table B.8, Appendix). Table 6 below contains the post-hoc results. 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 mono-unsaturated fatty acid, which were recorded.

Table 8. Post hoc Results of Fertilizer Effects on Polyunsaturated Fatty Acids (PUFA),
Monounsaturated Fatty Acids (MUFA), and Saturated Fatty Acids (SFA)

Treatment	Year	SFA	MUFA	PUFA	PUFA_ω6	PUFA_ω3	MUFA/PUFA	Oleic/Linoleinic
CONTROL	2022	9.14±0.125B*bc	71.183±0.19B*c	19.677±0.311B*bc	19.653±0.327B*b	0.03±0B*cd	3.618±0.067A*b	3.671±0.064A*b
	2023	10.92±0.284A*a	28.69±3.649C*a	60.39±3.831A*a	60.327±3.827A*a	0.06±0.01A*a	0.479±0.092B*a	0.476±0.092B*a
	2024	8.93±0.284B*a	84.667±2.665A*a	6.403±2.398C*a	6.333±2.389C*a	0.055±0.015AB*a	14.418±4.794AB*a	14.522±4.849AB*a
EM-1	2022	9.718±0.059B*ab	58.14±2.664B*d	32.143±2.726B*a	32.075±2.735B*a	0.05±0.005A*a	1.822±0.226A*c	1.816±0.227A*c
	2023	10.737±0.09A*a	31.005±0.978C*a	58.14±0.89A*a	58.09±0.89A*a	0.057±0.012A*a	0.533±0.025B*a	0.532±0.025B*a
	2024	8.7±0.106C*a	87.597±1.31A*a	3.707±1.34C*a	3.627±1.342C*a	0.09±0.05A*a	25.953±9.851AB*a	26.173±9.56AB*a
GOIM	2022	9.253±0.095B*abc	73.423±0.177B*bc	17.327±0.265B*bc	17.29±0.262B*bc	0.03±0C*c	4.238±0.074A*a	4.226±0.074A*a
	2023	11.03±0.305A*a	30.593±1.435C*a	58.37±1.256A*a	58.317±1.25A*a	0.057±0.006A*a	0.525±0.035B*a	0.522±0.035B*a
	2024	9.19±0.323B*a	85.597±1.384A*a	5.207±1.677C*a	5.167±1.677C*a	0.043±0.006B*a	18.043±7.466AB*a	18.129±7.572AB*a
GOIM +EM-1	2022	9.24±0.377B*abc	80.037±1.749B*a	10.72±1.382B*d	10.683±1.38B*d	0.04±0B*b	7.558±1.077A*ab	7.549±1.08A*ab
	2023	10.8±0.201A*a	29.21±3.212C*a	59.99±3.412A*a	59.93±3.402A*a	0.06±0.01A*a	0.49±0.08B*a	0.487±0.08B*a
	2024	8.637±0.319B*a	86.65±1.578A*a	4.71±1.333C*a	4.67±1.333C*a	0.04±0B*a	19.36±5.131A*a	19.45±5.19A*a
K	2022	9.023±0.156B*c	76.65±0.891B*ab	14.327±1.047B*cd	14.29±1.042B*cd	0.037±0.006B*bc	5.373±0.465A*ab	5.358±0.462A*ab
	2023	11.27±0.209A*a	28.403±3.562C*a	60.327±3.548A*a	60.27±3.543A*a	0.057±0.006A*a	0.474±0.086B*a	0.471±0.086B*a
	2024	8.647±0.191B*a	86.413±1.38A*a	4.937±1.534C*a	4.84±1.569C*a	0.045±0.005AB*a	18.576±5.182A*a	18.938±5.424A*a
K+EM-1	2022	9.267±0.304B*abc	74.347±1.099B*bc	16.387±0.897B*bc	16.347±0.897B*bc	0.04±0B*b	4.549±0.325A*ab	4.536±0.324A*ab
	2023	11.177±0.265A*a	28.563±4.227C*a	60.26±3.972A*a	60.197±3.966A*a	0.06±0.01A*a	0.478±0.1B*a	0.475±0.1B*a

	2024	8.907±0.652B*a	87.215±2.195A*a	4.19±1.83C*a	4.15±1.83C*a	0.04±0B*a	24.275±12.253AB*a	21.501±10.399AB*a
K+GOIM	2022	9.782±0.153B*a	58.19±1.141B*d	32.028±1.285B*a	31.978±1.28B*a	0.05±0.005B*a	1.82±0.107B*c	1.813±0.106B*c
	2023	11.053±0.136A*a	29.593±1.533C*a	59.357±1.415A*a	59.297±1.415A*a	0.06±0A*a	0.499±0.038C*a	0.496±0.038C*a
	2024	8.763±0.106C*a	87.17±1.062A*a	4.067±0.974C*a	4.027±0.974C*a	0.04±0C*a	22.251±5.134A*a	22.383±5.214A*a

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. The star symbol (*) denotes the statistical interaction between two factors (Year*Fertilizers treatment)

The subsequent multivariate two-way ANOVA tests of polyunsaturated PUFA demonstrated a significant year effect $F_{(6,42)}$ =3866.77, $p<0.001$, a treatment effect $F_{(6,42)}$ =13.345, $p<0.001$, and a significant interaction effect of year and fertilizers treatment $F_{(2,6)}$ =18.743, $p<0.001$ (Table B.8, Appendix). Table 6 displays the post-hoc test results obtained through pairwise comparison using Games-Howell's method. 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 (Games-Howell). 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 follow-up multivariate two-way ANOVA tests of polyunsaturated SFA revealed a significant year effect ($F_{(6,42)}$ =395.303, $p<0.001$) and a significant interaction effect of year and fertilizer treatment ($F_{(2,6)}$ =2.749, $p=0.008<0.05$) (Table B.8, Appendix). Pairwise comparison post hoc test results gained by using Games-Howell's method are shown in Table 6. 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

89

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 (Table 6).

4.4. Treatment Effects on Agronomic Traits in Sunflower crops over three years using heatmap Figure

The heatmap (Figure 30) illustrates the impact of various treatments on the growth and physiology of sunflowers over a range of years. The impact of treatments on the measured parameters (plant height, LAI, leaf number, stem diameter, head diameter, fluorescence, SPAD values, yield, seed weight, TKW, head weight, SN/head) is represented by each cell in comparison to the control. The magnitude and orientation of the change are indicated by the color ingredient. Darker shades denote bigger increases, while green or yellow indicate positive or greater changes. The use of red denotes negative or lower changes, while darker hues represent larger decreases. Small or no change is represented by white or pale hues. The dark green cells indicated that the treatment K+GOIM had a substantial positive effect on the change in head weight (+267.09) and the change in seed weight (+154.82g). The Change in Yield (+32.65 t/ha) and change in LAI (+124.92) exhibit lesser positive or moderate changes. In comparison to other treatments, the treatment demonstrated relatively favorable results, particularly in terms of head weight, seed weight, and yield. The treatment EM exhibited a significant positive impact on the change in leaf number (+22.68) and LAI (+26.21). Nevertheless, the dark red cell indicates that it had a substantial negative impact on the change in seed weight (-18.19 g). The GOIM treatment demonstrated moderate positive changes, particularly in the areas of LAI (+23.06) and seed weight (+24.56 t/ha). Conversely, other variables, such as plant height (-9.26g), were negatively impacted. The treatment GOIM+EM exhibited some adverse effects, including a decrease in plant height (-11.66) and moderate positive alterations in seed weight (+31.28 g). Several variables, including the change in LAI (+14.13), the change in SN/head (+18.10), and the change in seed weight (+30.03g), exhibited moderate positive alterations in the treatment K. In general, the K+GOIM treatment had the most significant positive effects on critical variables such as yield, head weight, and seed weight, indicating that they are the most effective treatment.
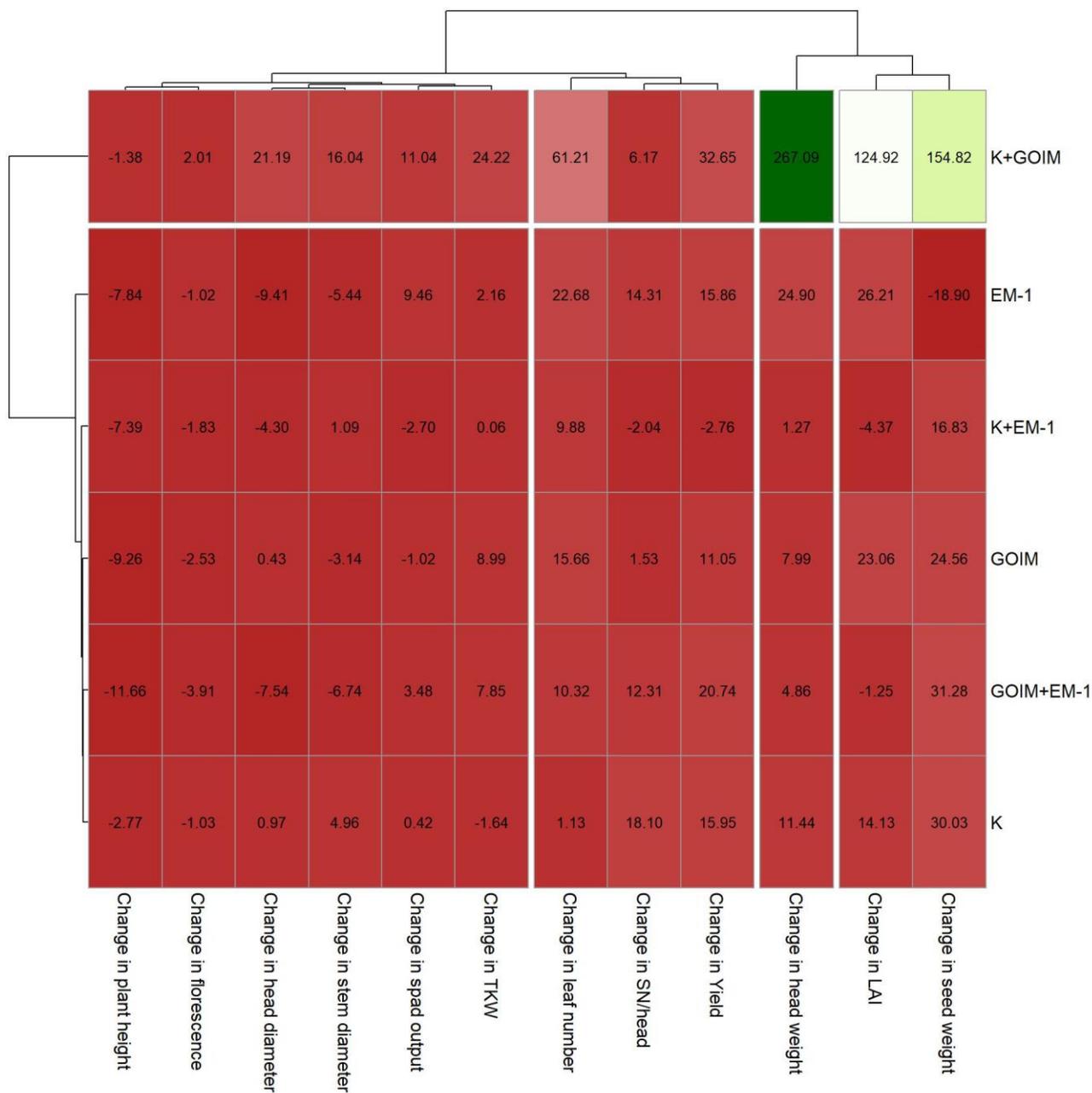
Figure 30. Heatmap of Treatment Effects on Agronomic Traits in Sunflower Crop

4.5.   Overall Impact of Fertilizer Treatments on Fatty Acid Composition in Sunflower Seeds Across Multiple Years using heatmap Figure
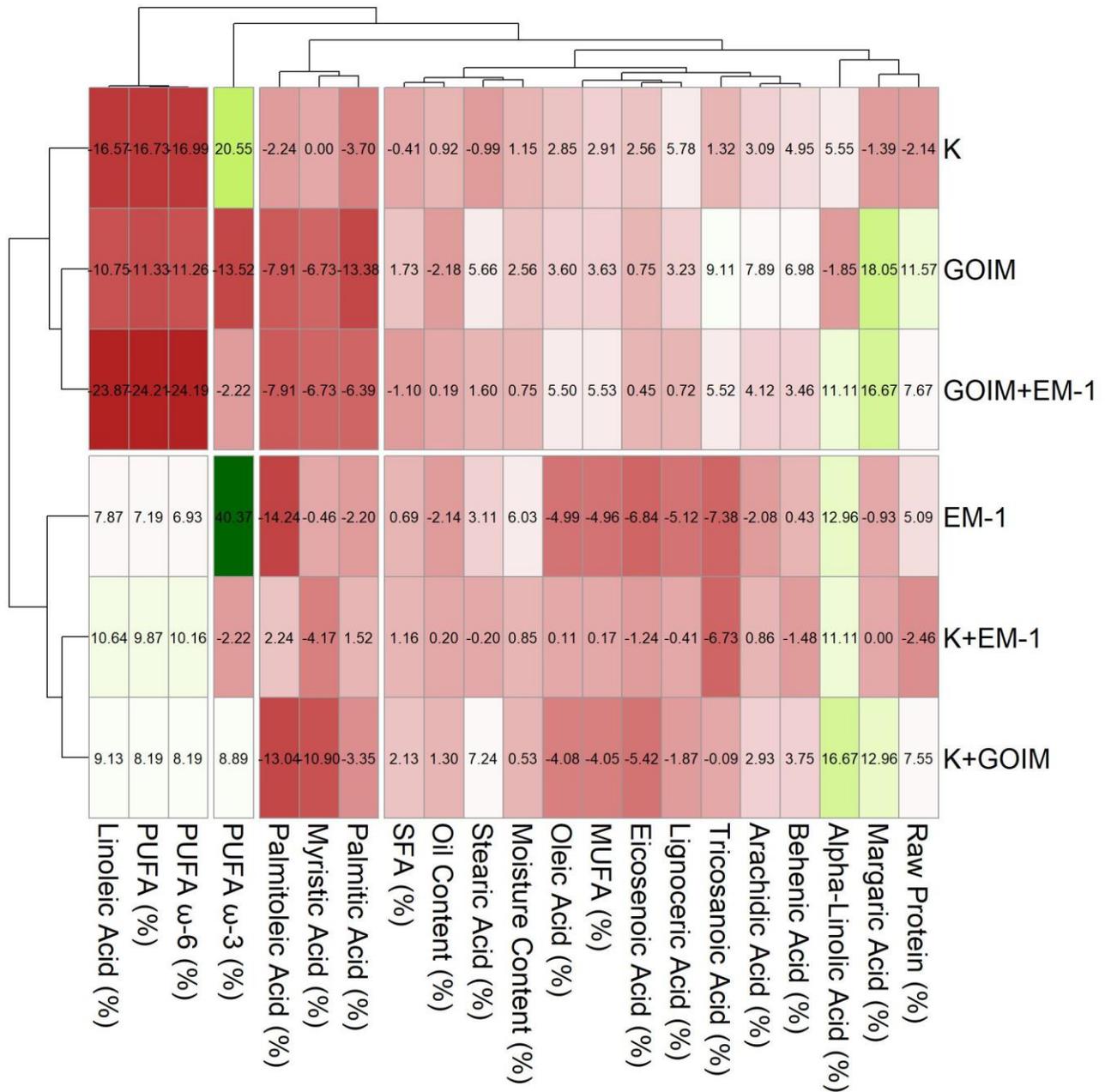
Figure 31. Heatmap of Treatment Effects on fatty acid composition in Sunflower crop

The heatmap (Figure 31) displays the impact of different fertilizer treatments on the percentage change of various fatty acids and oil components when compared to the control. Among the treatments analyzed are effective microorganisms (EM-1), potassium (K), organic nitrogen combined with inorganic nitrogen (GOIM), along with their combinations. Notably, the use of EM-1 on its own had

the most significant positive effect on polyunsaturated fatty acids (PUFA) ω-3 and alpha-linolenic acid, yielding increases of 40.37% and 12.96%, respectively, compared to the control (indicated by green areas) and a small increase of linoleic acid of 7.87%. Additionally, the change of K+GOIM compared to control increased alpha linoleic by 16.67%, PUFA (ω-3) by (8.89%), and linoleic acid by 9.13%. In contrast, the GOIM+EM-1 treatment experienced the greatest reductions in linoleic acid, PUFA, and PUFA (ω-6) with decreases of 23.86%, 24.21%, and 24.19% respectively (signified by red areas). Moderate variations were observed in oleic acid, an essential monounsaturated fatty acid, across the different treatments. Compared to the control, the oleic acid increased the most in oil from GOIM+EM by about 5.5% compared to the control. The K and K+EM showed similar results to GOIM+EM. However, GOIM+EM-1, K, and K+EM-1 increased alpha-linolenic acid, which is found in trace amounts in seeds. Overall, these results suggest that EM-1 is particularly effective in enhancing the levels of ω-3 fatty acids, while the combination of GOIM with other treatments tends to diminish the levels of ω-6 fatty acids and has a moderate impact on oleic acid.

4.6. AMMI PCA Analysis of Treatment Effects on Sunflower Growth and Yield Over Three Years

The response variable is significantly influenced by the year, fertilizers, and their interaction, as indicated by the AMMI (Additive Main Effects and Multiplicative Interaction) analysis. A $p<0.001$ indicates that the year effect has a highly significant influence, suggesting a significant variation in the response variable across various years. There is also a significant replicate effect within years (REP(Year)) ($p = 0.001574$), which implies that there is some variability among replicates within the same year. However, this effect is less pronounced than the main year effect (Table 9). Different fertilizer applications have a profound impact on the response variable, as evidenced by the exceedingly high F-value (2069.126) and a $p <0.001$. Additionally, the interaction between fertilizers and the year is highly significant ($p < 0.001$), indicating that the impact of fertilizers is dependent upon the specific year (Table 9). This interaction implies that the response to fertilizers is not consistent across years and that the environmental conditions unique to each year influence the impact of the fertilizers.

Table 9. ANOVA Results from AMMI Analysis of Fertilizer Treatments, Year, and Their Interaction on Sunflower Growth, Physiology, and Yield Traits

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	20	2488901	124445	9.3122	9.203e-10 ***
REP(Year)	42	561274	13364	1.8482	0.001574 **
Fertilizers	9	134650714	14961190	2069.1258	< 2.2e-16 ***
Year: fertilizers	180	9803525	54464	7.5323	< 2.2e-16 ***

The findings suggest that PC1 and PC2 jointly account for nearly all the variance in the dataset (99.9%), rendering them the most critical components for comprehending the data's patterns. PC3 through PC9 are statistically insignificant and contribute essentially no additional meaningful variance. This implies that the dataset can be effectively summarized and interpreted using only the first two principal components, which encompass nearly all the critical variation in the data (Table 10).

Table 10. Principal Component Analysis (PCA) Results from AMMI Analysis: Variance Contribution and Statistical Significance of PCs for Sunflower Traits

	Percent	Acum	Df	Sum.Sq	Mean. Sq	F.value	Pr. F
PC1	77.3	77.3	28	7.58E+06	2.71E+05	37.43	0
PC2	22.6	99.9	26	2219215	8.54E+04	11.8	0
PC3	0.1	100	24	6028.674	2.51E+02	0.03	1
PC4	0	100	22	482.8809	2.19E+01	0	1
PC5	0	100	20	1.53E+02	7.66E+00	0	1
PC6	0	100	18	18 9.552551e+01	5.31E+00	0	1
PC7	0	100	16	2.91E+01	1.82E+00	0	1
PC8	0	100	14	1.07E+00	7.68E-02	0	1
PC9	0	100	12	9.62E-02	8.01E-03	0	1

Figure 32. Biplot analysis of the growth, physiology and yield parameters in response to different fertilizer treatments and years using the AMMI model

Figure 32 is a Principal Component Analysis (PCA) biplot, a technique that is frequently employed to reduce the dimensionality of multivariate data and to illustrate the relationships between variables and samples. The y-axis represents the first principal component (PC1), which explains 77.3% of the variance, while the x-axis represents the second principal component (PC2), which explains 22.6% of the variance. These components collectively account for 99.9% of the variance in the dataset, thereby capturing the majority of the significant variation (Table 10). The arrows in the dataset represent various variables, including seed weight (g), which is positioned to the right, and SN/head, which is positioned further to the right. This relationship is strongly correlated with PC2. The head weight (g) is positioned to the left and is more influenced by PC1. The length of these arrows is indicative of the extent to which the variables contribute to their respective principal components, with longer arrows indicating more significant contributions. Sample points are individual observations in the dataset. Their position on the diagram is indicative of their relationship to the principal components and the variables. Samples that are closer together imply a higher degree of similarity, while those that are

farther apart indicate a more distinct behavior. The data clusters are illustrated by the arrangement of points according to treatments and years (2022, 2023, 2024), which offers unique perspectives on the distinctions between treatments and years. The treatments GOIM+EM-1-2023, EM-1-2023, K+EM-1-2023, and CONTROL-2023 are situated in close proximity to 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.

4.6.1. AMMI PCA Analysis of Treatment Effects on Fatty Acid Composition Over Three Years

The analysis results indicate that the fatty acid composition and oil quality are significantly influenced by both the year and fertilizer applications. The factor Year is statistically significant ($p = 0.001$) (Table 9), indicating that the measured parameters of oil quality are significantly influenced by the environmental conditions of various years. Furthermore, the replicates within each year also demonstrate highly significant differences ($p < 0.001$) (Table 11), suggesting that the variation among repetitions in the same year is not a result of arbitrary chance. This underscores the necessity of accounting for year-to-year variability in oil composition analysis. Furthermore, the fertilizer treatments are highly significant ($p < 0.001$) (Table 11), which confirms that the quality of the oil is substantially influenced by the type of fertilizer used. This finding suggests that specific fertilizers are more effective in improving oil composition than others, underscoring the significance of selecting the appropriate fertilizer types to enhance oil yield and composition. The interaction between fertilizer treatments and year is also highly significant ($p < 0.001$) (Table 11), suggesting that the impact of fertilizers on oil quality is dependent upon the unique conditions of each year. This interaction implies that the efficacy of fertilizers may fluctuate as a result of external factors, including soil conditions, weather, or other environmental influences. Consequently, optimizing oil quality outcomes necessitates appropriate fertilizer application strategies for the unique conditions of each year.

Table 11. ANOVA Results from AMMI Analysis of Fertilizer Treatments, Year, and Their Interaction on Sunflower oil and fatty acid composition

	Df	Sum	Sq Mean	Sq F value	Pr(>F)
Year	20	38127	1906.37	3.0882	0.001029 **
REP(Year)	42	25927	617.3	2.1659	3.76e-05 ***
fertilizers	20	19270	963.51	3.3806	8.96e-07 ***
Year: fertilizers	400	281018	702.55	2.465	< 2.2e-16 ***

The first two principal components (PC1 and PC2) together account for 87.3% of the total variance, suggesting that they encompass the majority of the variation in your data (Table 12).

Table 12. Principal Component Analysis (PCA) Results from AMMI Analysis: Variance Contribution and Statistical Significance of PCs for Sunflower Traits

Analysis	percent	acum Df	Sum.Sq	Mean. Sq	F.value	Pr. F
PC1	63.4	63.4 39	1.78E+05	4568.422	16.03	0
PC2	23.9	87.3	37 6.707096e+04	1812.729	6.36	0
PC3	6.4	93.7	35 1.810125e+04	517.1786	1.81	0.0031
PC4	2.2	95.9 33	6.22E+03	188.3712	0.66	0.9299
PC5	1.3	97.2 31	3.52E+03	113.4482	0.4	0.9987
PC6	0.9	98.0 29	2441.599	84.19308	0.3	0.9999
PC7	0.6	98.7 27	1.80E+03	66.68929	0.23	1
PC8	0.4	99.1 25	1.05E+03	41.89071	0.15	1
PC9	0.3	99.4 23	9.48E+02	41.20271	0.14	1
PC10	0.2	99.6 21	6.21E+02	29.56045	0.1	1
PC11	0.2	99.8 19	4.85E+02	25.54407	0.09	1
PC12	0.2	99.9 17	4.27E+02	25.12933	0.09	1
PC13	0	100.0 1	1.08E+02	7.176772	0.03	1
PC14	0	100	13 5.007658e+01	3.852045	0.01	1
PC15	0	100	11 9.276986e+00	0.843362	0	1
PC16	0	100	9 4.212810e+00	0.46809	0	1
PC17	0	100	7 2.313471e+00	0.330496	0	1
PC18	0	100	5 2.089950e-01	0.041799	0	1
PC19	0	100	3 0.000000e+00	0	0	1
PC20	0	100	1 0.000000e+00	0	0	1

The AMMI model's biplot analysis of the oil quality parameters in response to various fertilizer treatments and years provided valuable insights into the interactions between treatment and year(Figure 33). Principal Component 1 (PC1) accounted for 63.4% of the total variance, while Principal Component 2 (PC2) accounted for 23.9%. Combined, these components accounted for 87.3% of the total variability in the dataset. This implies that these two principal components captured the majority of the variability in the oil fatty acid.
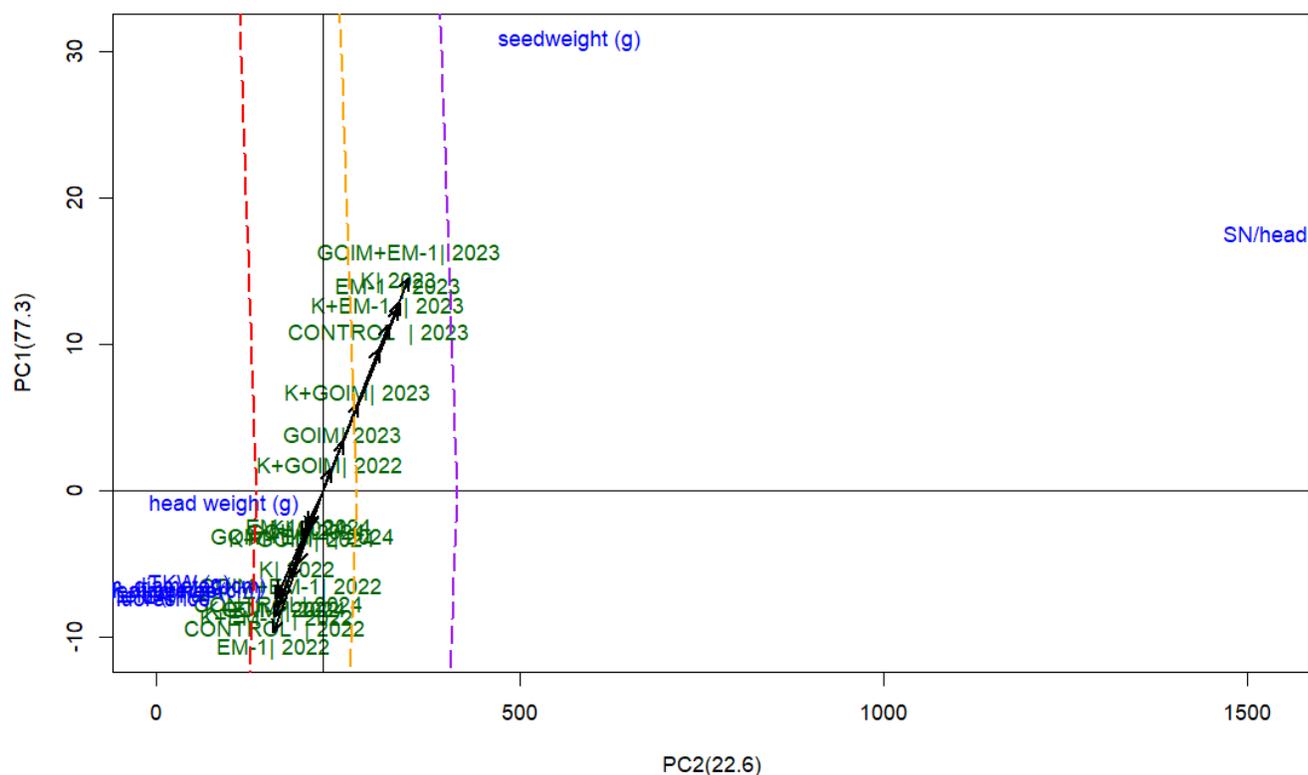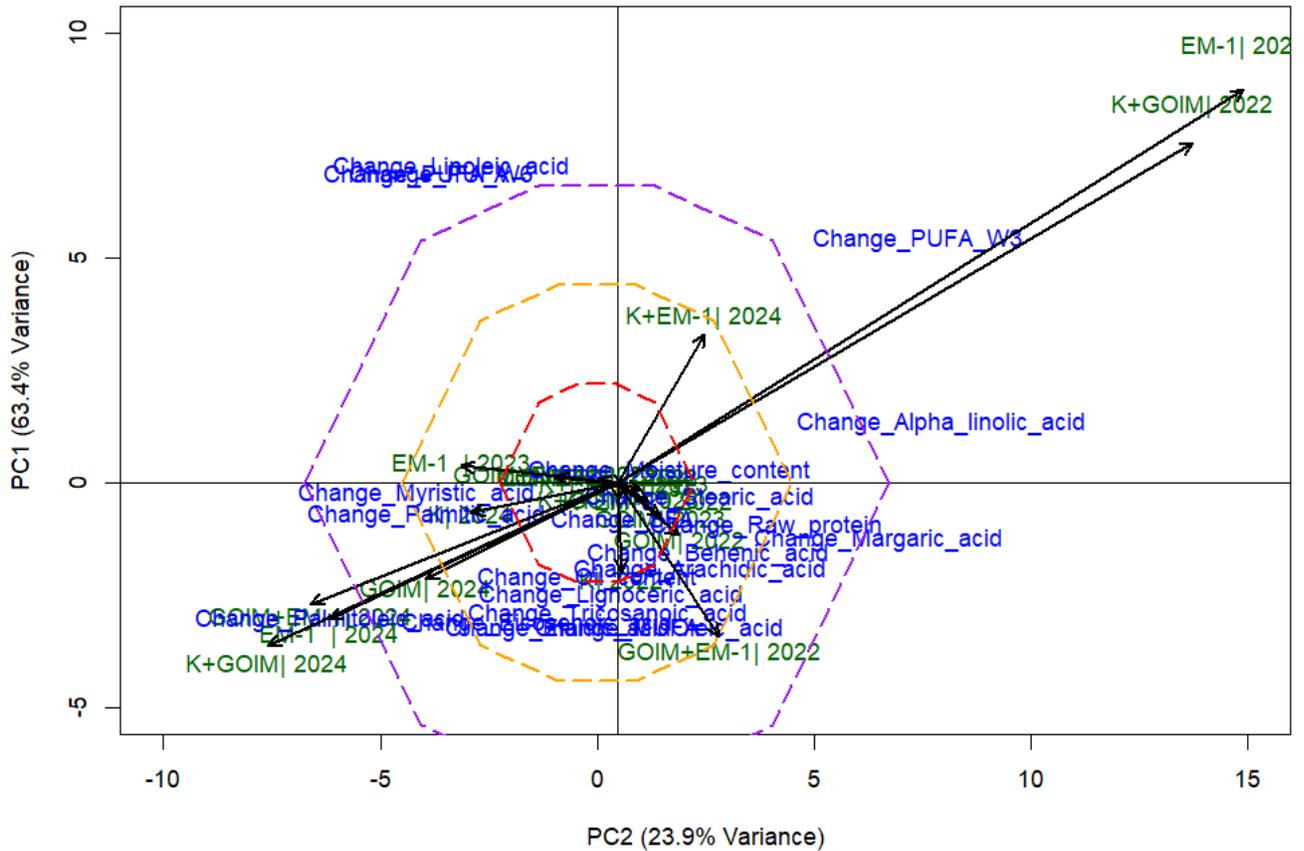
Figure 33. Biplot analysis of the oil quality parameters in response to different fertilizer treatments and years using the AMMI model

The contour lines help visualize the clustering of different fertilizer-year combinations. For instance, treatments within the same contour lines exhibit similar effects on oil quality parameters, while those falling outside of these lines display more distinct influences. This suggests that the specific year and type of fertilizer can lead to similar or divergent effects on oil composition depending on the treatment. The results (Figure 33) indicate that the polyunsaturated fatty acid (PUFA ω-3) and alpha-linoleic acid contents of the critical oil quality traits were significantly and positively influenced by the combination of K+GOIM fertilizer in 2022 and EM-1 fertilizer in 2022. This treatment is positively correlated with an increase in these beneficial oil components, which are crucial for the nutritional quality of oil, as indicated by the direction and magnitude of the arrows for these parameters. In comparison to other fertilizer-year combinations, the significant impact of these fertilizer treatments on enhancing oil composition is evident in their position far from the center of the biplot in 2022. However, treatments such as GOIM in 2024 and EM-1 in 2024 were positioned closer to the center of

the biplot, indicating that these combinations had a more moderate or balanced effect on oil quality parameters. These Treatments may not result in substantial changes in the measured oil characteristics, but rather in the preservation of a consistent oil quality profile. The results suggest that the most promising treatments for improving oil quality in 2022 are the combination of K+GOIM and EM-1 fertilizers, particularly in terms of increasing the levels of polyunsaturated fatty acids such as PUFA ω-3. This implies that the nutritional quality of oils can be substantially enhanced by the optimal application of fertilizers and favorable environmental conditions during specific years. These results emphasize the significance of selecting the appropriate fertilizer treatments in conjunction with environmental factors to optimize oil composition. Additional research should examine the mechanisms that underlie these enhancements, as well as the long-term impacts of these fertilizer applications on oil quality and crop yield.

5. DISCUSSION

5.1. Discussion of Germination traits

5.1.1. Effect of temperature levels on germination

The germination experiment showed that the optimal temperature range for sunflower seed germination was determined to be between 15°C and 30°C. Germination rates and speeds were both elevated within this range. Nevertheless, the substantially longer germination time at 15°C suggests reduced metabolic activity, signifying suboptimal efficiency, despite the fact that the maximum germination percentage (75%) was recorded. Conversely, the germination rate was marginally lower at 30°C, but it was significantly faster, indicating that it is the most effective temperature for sunflower germination in terms of both speed and efficacy. Germination was delayed and success rates were reduced at temperatures below 15°C, while germination was sped up at temperatures above 30°C (e.g., 35°C and 40°C), resulting in fewer overall percentages. The most favorable germination conditions were therefore observed between 15°C and 30°C. The germination process was delayed by low temperatures (5°C and 10°C) as a result of cold stress and reduced metabolic activity. Conversely, high temperatures (35°C and 40°C) resulted in a decrease in germination rates, which was likely due to heat stress and physiological harm. The seeds' ability to germinate effectively is restricted by these extremes. In general, sunflower seeds demonstrate the optimum productivity within the temperature range of 15°C to 30°C. This finding is supported by other research, which has indicated that the optimal temperature is approximately 25 °C (GAY et al., 1991; Khalifa et al., 2000). A higher germination rate is feasible at the optimal temperature (ALVARADO and BRADFORD, 2002). Furthermore, it facilitates a more effective combination of germination rate and percentage (MARCOS FILHO, 2015). In another study, it was reported that sunflower seeds do not germinate well at 10°C, but they can completely germinate at 20°C (XIA et al., 2019). Temperatures of 5°C or lower result in delayed germination and necessitate an extended period. The process of germination is expedited by elevated temperatures (CASSARO-SILVA, 2001; WINDAUER et al., 2012). Essential pathways for germination initiation cease to function at critical high and low temperatures, which reduce the metabolic rate. It may result in inadequate seedling development and a reduction in seed viability. Additionally, the increased metabolism of plants results in the depletion of the seed energy necessary for growth during periods of high temperatures (HAWKER and JENNER, 1993). Temperature stimulates metabolic and enzyme activity, as well as the seed's capacity to assimilate

water, thereby improving germination (OLIVEIRA et al., 2013). Germination is prevented by the rapid loss of available energy needed for growth at exceedingly high temperatures (CASSARO-SILVA, 2001). The germination rate is reduced, and the latency time to the onset of germination is prolonged as a result of high temperatures, particularly those around 44.3°C (CATIEMPO et al., 2021). The MGT decreased from 8.98 days to 1.43 days, and the germination time decreased from 5°C to 30°C. Furthermore, the germination rate was enhanced within the optimal temperature range. Our research is consistent with other investigations that have investigated the impact of temperature on different crops, like cotton. They discovered that germination speed increased by 61% at ambient temperatures (32-38.5°C) in comparison to 25°C (SINGH et al., 2018). The germination speed and percentage were influenced by a variety of temperature ranges, ranging from 5°C/1°C to 30°C/15°C. Some plants exhibited rapid germination at moderate temperatures (CHEN et al., 2019). Despite the fact that the final germination percentage was similar, seeds germinated at 21°C up to 21 times faster than at 5°C when given sufficient time(CHEN et al., 2019).

5.1.2. Effect of temperature levels on seedling development of Sunflower

The temperature is necessary for plants to transition from one phase of their life cycle to the next. The optimal temperature for seedling growth was determined by subjecting seeds to eight constant temperature levels, which ranged from 5 to 40°C, in this study. Seedling growth, when radicle reaches 1 cm, commenced at a temperature of 25–30°C. The initiation of germination was progressively delayed below these temperatures. The optimal temperature for germination was 25°C, with a range of 15–35°C, and sunflower seeds germinated at temperatures ranging from 5°C to 30°C in the current study. The existence of a high- and low-cut threshold between the temperature range of studies is suggested by a substantial decrease in germination potential and germination energy at or above 25°C. The optimal temperature range for sunflower germination and seedling development was established to be 25°C, with a range of 15 to 35°C. According to HAWKER and JENNER (1993), the seeds' maturation was expedited by the high temperature of 40°C, as evidenced by the fact that the majority of enzymes were rendered inactive and the seeds perished.

5.1.3. Effect of water levels on seedling development of sunflower

A prerequisite for germination is the absorption of water. The potential for seed germination was significantly influenced by the quantity of water. With the one-milliliter intervals technique and TKW

water levels, sunflower seeds can germinate at low and high-water availability, starting at 0.6 ml or 125%, according to both methodologies. This is due to their dense and long roots. This quantity may be equivalent to or nearly identical to the moisture content necessary for germination. According to the literature, the estimated proportions for maize, wheat, and soybeans are 30%, 40%, and 50%, respectively (VIERLING and KIMPEL, 1992). These variations may be attributable to species diversity. Once the critical seed moisture content is achieved, the presence of adequate moisture is sufficient to trigger germination. This process initiates a sequence of physiological modifications within the seed, such as the mobilization of stored nutrients and the activation of enzymes. During the plateau phase of seed imbibition, which is a critical phase in the regulation of seed germination, the minimum water potential required to induce seed imbibition has been documented in previous research (CAMPOS et al., 2020; RAMÍREZ-TOBÍAS et al., 2014). It was noted that the duration of this phase is significantly influenced by the species and water potential. In certain species, it can be prolonged to water potentials of approximately -0.03 MPa (RAMÍREZ-TOBÍAS et al., 2014), while in others, it can be extended to -1.0 MPa (CAMPOS et al., 2020). It is crucial to comprehend the species-specific responses to hydration conditions, as this variability can have a substantial influence on the overall establishment of plants and the rate of germination. The seedling's growth is facilitated by the availability of water. The optimal water range for total seedling length was 8.2 to 11.4 ml, which corresponded to 1625 to 2250% of TKW. This range was equivalent to radicle and plumule (HAJ SGHAIER et al., 2023). Significant differences were observed in the dry weight accumulation of the seedlings under varying water conditions (Tables 5 and 6). The TKW method is accurate in determining the minimum optimal water requirement for germination, as evidenced by previous research (HAJ SGHAIER et al., 2022; KHAEIM et al., 2022a, 2022b). The optimal range for the seedling dry weight was 4.4–8.4 ml, which was lower than the optimal water for seedling growth. The water ranges necessary for optimum seedling growth and maximum dry weight are significantly different. The dry weight range is less extensive than that for general growth. This may suggest that an excessive amount of water can result in larger seedlings that are potentially less dense or weakened, while a lack of water promotes denser, more compact growth, as evidenced by a higher dried weight. The optimal water levels should be selected in accordance with the specific objectives of seedling development in future research. A higher water range (8.2–11.4 ml) appears to be more effective in promoting rapid and vigorous seedling growth. Nevertheless, a lower water range (4.4–8.4 ml) is more suitable for the purpose of generating seedlings that are more compact and resilient, as evidenced by a higher dry weight. It is crucial to acknowledge that these discoveries were derived from Petri dish

experiments and may not accurately reflect real-world conditions. Water availability and seed–soil contact are substantially influenced by soil structure, capillarity, and field water dynamics, which can affect germination and growth outcomes in natural or agronomic environments.

5.1.4. Effect of seed number density on seed germination and seedling growth

The objective of this experiment was to ascertain the optimal number of seeds that would yield the maximum germination efficiency by comparing the effects of 6, 8, 10, and 12 sets of seeds per PD on germination and growth parameters. The seed numbers set varied significantly in terms of the growth and dry weight of the radicle and shoot. As the number of seeds per Petri dish increased, the desiccated weight and seedling growth also increased. A set of 12 seeds per PD was employed to observe high seedling length and dried seedling matter. Although the germination rate was improved by seed densities of 6–8, the development of the seedlings was slowed. This finding may be attributable to the shortage of a vital resource, such as water, at a low seed number as a result of competition (CALLAWAY and WALKER, 1997; WHITE, 2001). In addition, dense seedlings could be more susceptible to lodging, which increases disease incidence (GLEN et al., 1989) and, as a result, the seedling emergence percentage (LORTIE and TURKINGTON, 2002). Under controlled conditions, the ideal seed density for sunflower cultivation in vitro is 12 seeds per PD (see Table 4). Consequently, it is essential to optimize seed density by PD and other environmental factors during germination experiments.

5.1.5. Effect of antifungal experiment on seedling development

As many researchers have suggested in their works, the absence of fungi in our experiment may have been the primary factor, rather than the phytotoxicity of the fungicide (VASANTHAKUMARI et al., 2019; VERMA et al., 2018, 2017). The primary function of the antifungal Amistar Xtra is to inhibit fungal growth, which harms the seedlings. These results are consistent with previous research (HAJ SGHAIER et al., 2023, 2022; KHAEIM et al., 2022a, 2022b; TARNAWA et al., 2023). It contains the active component azoxystrobin, which impedes electron transport in the mitochondrial respiratory chain of fungi, thereby reducing aerobic energy production and delaying the growth of fungi (BERTELSEN et al., 2001). The use of fungicide as a control of fungal growth or pretreatment of sunflower seeds resulted in a dose-dependent decrease in seedling growth, with the highest concentration proving severely phytotoxic, nearly killing the seedlings. This suggests that Amistar,

while effective as a fungicide, can be highly detrimental to seedling vigor when applied directly to seeds under in vitro conditions. These findings suggest that Amistar is not suitable for direct seed disinfection prior to fungal growth control, to in vitro germination, and emphasize the need to assess safe application protocols for fungicides in early developmental stages. Furthermore, they observed a resurgence in seedling growth after inoculation and fungal exposure. Treatment with 10% sodium hypochlorite (NaClO), commonly referred to as Hypo, showed no significant difference from the control in terms of seedling growth and germination performance. This indicates that NaClO was effective for surface sterilization without inducing phytotoxic effects on the seeds. The chemical likely acted externally, reducing potential microbial contamination while preserving seed viability. Unlike systemic fungicides, NaClO does not penetrate deeply into seed tissues, thus avoiding disruption of internal physiological processes. These findings support the use of hypochlorite (NaClO) as a safe and reliable seed disinfectant in controlled in vitro germination studies.

5.2.   Discussion of Fertilization traits

The influence of various fertilizer treatments on sunflower cultivars was assessed over a three-year period in this study, which revealed variations in their effects on oil quality, yield, physiology, and growth. The significance of ambient factors in determining the efficacy of these treatments is underscored by the year-to-year variations. The year 2023 had the highest precipitation, which coincides with the vegetative and seed-filling phase which followed by the years 2024 and 2022. In the experimental field, 2022 was the wettest year.

5.2.1. Effect of fertilizers treatment on growth and physiology of sunflower crops

Growth and physiological responses to fertilizers did not significantly influence the height of the plant that failed to pass through. Nevertheless, the K+GOIM supplemented the control plant's height in comparison to other fertilizer treatments. This is in line with earlier studies' conclusions that applying potassium and organic manure (such as chicken or cattle manure) together can greatly increase sunflower plants' height (BURIRO et al., 2015a, 2015b; BYRAREDDY et al., 2010; DAMBALE et al., 2018b; FARHAD et al., 2020; SAYED et al., 2022; SEFAOGLU et al., 2021). Nonetheless, it is crucial to acknowledge that in our instance, the control consistently yielded the highest results. The impact of fertilizers extends beyond plant height; notable enhancements in structural development were also recorded, evidenced by the substantial rise in stem diameter in the K, GOIM, and K+GOIM

treatments. The K and GOIM, either alone or collectively, augmented the stem diameter. An increased stem diameter enhances the plant's vascular system, hence augmenting its capacity to carry water and nutrients effectively. This is essential for maintaining elevated productivity, particularly under fluctuating climatic circumstances like drought. Similar results were noted by BURIRO et al. (2015b), who found that treatments of potassium and nitrogen considerably enlarged the stem diameter of sunflower plants, hence improving the transfer of nutrients and water. In comparison to control groups, the application of NPK, which contains potassium, alongside organic manure, produced taller plants with thicker stems, enhancing overall plant structure. The K+GOIM treatment not only facilitated structural growth but also resulted in notable enhancements in the LAI. This enhanced canopy structure enables plants to capture more light for photosynthesis, hence enhancing biomass and production. 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. Comparable findings were documented by (DAMBALE et al., 2018b; ELANKAVI, 2017b), who discovered that the amalgamation of organic and inorganic nitrogen with potassium increased canopy growth in sunflower crops by facilitating effective nutrient absorption. Effective Microorganisms (EM) and K+GOIM treatments are essential in this case because they increase nutrient availability, which enhances leaf development and overall plant vigor. 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. This result corresponds with previous observations of (ARIF et al., 2016) and (SEFAOĞLU, 2021), who reported enhancements in chlorophyll content and several growth metrics in nitrogen-enriched compost supplemented with mineral nitrogen fertilizers. Nonetheless, our research did not align with the investigation conducted by ALZAMEL et al. (2022), which examined the impact of inorganic fertilizers, compost combined with biofertilizer, and filter mud cake on the quality of sunflower oil. The findings indicate that inorganic fertilizers were superior in boosting plant growth and oil quality characteristics, such as chlorophyll content and phenolic compounds, but organic fertilizers significantly contributed to increasing seed and oil yields. Furthermore, the study by MANIKANDAN

and THAMIZHINIYAN (2017) demonstrated that organic fertilizer is more effective than inorganic fertilizer over control. Similar results were observed in treatments involving EM (ABU-QAOUD et al., 2021; EL-KHATEE et al., 2018; IRITI et al., 2019; JIANG et al., 2024; TALAAT, 2019; YOUSSEF et al., 2021), who noted that effective microorganisms augment chlorophyll content and photosynthetic efficiency, thereby promoting plant growth, yield, and nutrient absorption. The results demonstrate that measures including LAI, leaf number, stem diameter, and SPAD values surpassed those of the control, despite plant height not exhibiting the same trend. This result may be ascribed to several reasons. The application of fertilizers may have improved leaf development, stem thickness, and chlorophyll content without necessarily augmenting vertical growth. Plants frequently emphasize lateral development and leaf expansion when nutritional circumstances are favorable, particularly with the availability of potassium (K) and nitrogen (N). Furthermore, environmental stressors, such as the 2022 drought, may have driven plants to allocate greater resources towards fortifying stems and enlarging leaf surfaces to enhance water retention and photosynthetic efficiency, rather than prioritizing vertical growth. The fertilizers, especially potassium and nitrogen, probably influenced the physiological characteristics of the plant more directly, including chlorophyll synthesis and stem robustness, rather than stem elongation. This is evident in the elevated Leaf Area Index (LAI), leaf number, and SPAD measurements. Sunflowers may possess a genetic growth pattern in which specific nutritional circumstances promote biomass accumulation in leaves and stems, resulting in enhanced overall development but restricted height growth. The fertilizers, particularly K+GOIM, positively influenced structural and physiological growth, despite plant height being comparable to the control group.

5.2.2. Effect of fertilizer treatment on yield attributes of sunflower crop

The study's findings demonstrate that various fertilizer treatments significantly and diversely influenced sunflower production characteristics across many years. The treatment, including potassium, organic nitrogen, and inorganic nitrogen (K+GOIM), consistently yielded the greatest values, especially in 2022. The driest year during the experimental investigations. This indicates that the synergistic interaction of potassium with both organic and inorganic nitrogen establishes ideal growth circumstances, improving critical metrics such as head weight, head diameter, seed weight, and TKW. The combination of cow manure with inorganic fertilizer yields a synergistic enhancement in crop performance (ESMAEILIAN et al., 2012). They reported that combining organic nitrogen, which provides a steady release of nutrients, with inorganic nitrogen, which offers quick availability,

resulted in higher sunflower yields compared to inorganic fertilizers alone. This supports the current study's observation that the K+GOIM treatment yielded the highest parameters, especially in 2022, the driest year of the experiment, suggesting its critical role in drought resilience. Potassium significantly enhanced these traits by improving water uptake and stomatal function, especially in water-limited environments. Furthermore, the results of this study are consistent with earlier research that emphasizes the substantial impact of applying the recommended dosage of fertilizer and farmyard manure (BHATTACHARYYA et al., 2008; DAMBALE et al., 2018b; JANMOHAMMADI et al., 2016a; NANJUNDAPPA et al., 2001). The research by SAYED et al. (2022) demonstrated that a foliar spray of Potassium Nitrate at a concentration of 3%, along with 14 tons/ha of Poultry Manure under water stress circumstances, was optimal for growth and yield characteristics. Moreover, MUNIR et al. (2007) demonstrate that the use of a specific amount of NPK fertilizer (50-75-50 kg/ha) in conjunction with the integration of chicken manure at 8 tons/ha markedly increases the production of spring sunflower crops and promotes the effectiveness and consumption of inorganic fertilizers. This integration improves photosynthesis by augmenting leaf area and chlorophyll concentration, hence enhancing total biomass and seed development. The availability of nutrients, especially during the reproductive period, enhances the development of bigger, well-filled sunflower heads with increased seed and oil content. Furthermore, organic matter helps retain moisture, and potassium improves drought tolerance, making it possible for sunflowers to tolerate less-than-ideal water circumstances. Organic matter enhances soil structure and microbial vitality, facilitating more effective nutrient absorption, especially of nitrogen and potassium, which are crucial for optimal development and productivity. The GOIM+EM treatment, incorporating beneficial microorganisms, demonstrated superior yield characteristics, especially in 2023. The environmental conditions of 2023 may be more conducive to the integration of organic and inorganic matter, as well as effective microorganisms, to achieve high yield parameters. This underscores the significance of EM in augmenting soil health, optimizing nutrient absorption, and promoting root development, resulting in improved crop production when combined with farmyard manure and NPK fertilizer (JAVAID and BAJWA, 2011). Similarly, combining EM with compost significantly increased wheat yields. By promoting healthy plant growth, EM contributes to higher crop yields and improved crop quality (YOUNAS et al., 2022). In a study by KHALIQ et al. (2006), the integrated use of organic materials and effective microorganisms (EM) significantly increased cotton yield by 44% compared to the control group. The application of NPK fertilizers in conjunction with organic materials and EM resulted in the maximum seed cotton yield. Furthermore, a yield that is comparable to that of a full

NPK application is achieved by combining half the recommended dosage of mineral NPK with EM and organic materials. Our research also verified that, although EM improves nutrient efficacy, it is ineffective in boosting yields on its own. The GOIM alone increased yield parameters in 2024, suggesting that, despite the effectiveness of organic-inorganic nitrogen combinations, the addition of EM can provide an additional rise in certain years, likely dependent on environmental factors such as soil microbial activity or moisture availability. Soil microbial activity assessments would provide valuable insights into the mechanisms by which EM-1 and other fertilizers contribute to drought resilience and soil health. 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. The EM functions as biofertilizers to improve the availability and assimilation efficiency of nutrients and increase crop productivity(PARAMESH et al., 2023). Our results are additionally corroborated by the research of (MUKHERJEE et al., 2019; RAJ and MALLICK, 2017). The leaf area index, dry matter accumulation, seed yield, oil content, and positive impacts on soil fertility status are all improved by the combination of 50% RDF, poultry manure, and biofertilizers (*Phosphate-solubilizing bacteria* and *Azotobacter*). According to a study conducted by MALLICK and MAJUMDER (2022), the most effective method for optimizing seed yield and dry matter production was the combination of organic manure, vermicompost, biofertilizers, and 50% of the recommended inorganic fertilizers, which was supplemented with foliar application.

5.2.3. Impact of Fertilizer Applications on Nutritional Composition (Moisture, Oil, and Protein) of Sunflower Seeds

The observed increase in oil content in the K+EM and K treatments corresponds with potassium's function in enhancing fatty acid production and oil accumulation in sunflower seeds. Potassium, in conjunction with a balanced supply of effective microorganisms, facilitates essential metabolic processes associated with oil production. To our knowledge, no study has examined the interaction between K and EM in sunflower cultivation. Our findings confirm those of previous research, not in sunflowers, but in different crops. The integration of microorganisms, including *Azotobacter Chroococcum* and *Arbuscular Mycorrhizal* fungi, with chemical fertilization in safflower production markedly enhanced oil content and yield components. The highest oil content was recorded when these microorganisms were utilized in conjunction with chemical fertilizers, demonstrating a synergistic impact between microbial inoculants and chemical inputs (MIRZAKHANI et al., 2014).

Similar to this, using chemical fertilizers in conjunction with biofertilizers (*Mycorrhizae* and *Thiobacillus*) increased the oil content and yield of soybeans. The mycorrhizal treatment demonstrated a significant enhancement in oil content, highlighting the beneficial effect of microbial inoculants on soybean oil production (MOSTAFAVIA et al., 2008). Effective Microorganisms (EM) enhance potassium use efficiently in plants, as demonstrated by the significant rise in potassium content following EM treatment. This improved nutrient absorption facilitates superior plant growth and development (JAVAID and BAJWA, 2011). Furthermore, research indicates that the synergistic use of EM and potassium fertilizers results in increased yields across several crops. This combination of crops, such as tomatoes and mung beans, led to enhanced yields and superior nutritional content, underscoring the synergistic influence of EM and potassium on the overall growth of plants (JAVAID and BAJWA, 2011; KLEIBER and SIWULSKI, 2014). The use of potassium enhanced the quality of sunflower oil by elevating the levels of beneficial fatty acids, including oleic acid and linoleic acid. These fatty acids enhance the nutritional and commercial value of the oil (BABAR et al., 2024; LI et al., 2018). Moreover, under drought stress circumstances, potassium fertilization preserved superior oil quality by improving biochemical characteristics and seed components, so assuring that sunflower seeds yielded healthy, high-quality oil even in challenging conditions (ZAMANI et al., 2020). Nonetheless, the EM and K+GOIM treatments enhanced the moisture content of sunflower seeds. This suggests that these treatments establish ideal circumstances for seed development, likely to enhance nutrient availability and soil moisture retention. The rise in moisture content can be associated with enhanced water absorption and retention in the seeds, which is essential for preserving seed quality and viability during storage. EM can enhance plant resilience and productivity in stressful environments (HUSSEIN, 2024). Moreover, a study revealed that the simultaneous foliar application of nitrogen and potassium enhances physiological indices and metabolic processes in sunflowers, resulting in increased protein content under water deficiency circumstances (HUSSAIN et al., 2016). This combination aids in preserving turgor, augmenting Osmo protectant accumulation, and elevating net photosynthesis, so jointly raising protein yields. The increase in raw protein content in the K+GOIM (potassium + 50% nitrogen + 50% organic matter) and GOIM+EM (50% nitrogen + 50% organic matter + effective microorganisms) treatments is due to the synergistic effects of nutrient management and microbial activity. The application of 50% nitrogen, an essential macronutrient for protein synthesis, is vital for enhancing amino acid biosynthesis, the fundamental components of proteins. These treatments facilitate the integration of nitrogen with organic matter, ensuring a gradual release of nitrogen that offers a steady supply throughout the plant's development cycle, so promoting

protein synthesis. Organic matter strengthens soil structure, increases microbial activity, and facilitates nutrient availability, hence improving nitrogen absorption and use (AMANULLAH and KHAN, 2011). The GOIM+EM treatment enhances nitrogen availability through effective microorganisms that facilitate nitrogen fixation, nutrient solubilization, and promote root development. These bacteria enhance metabolic processes related to protein synthesis, consequently augmenting the raw protein content in sunflower seeds. In the K+GOIM treatment, potassium is essential for activating enzymes involved in protein synthesis and enhancing nutrient transport inside the plant. The presence of potassium, in conjunction with organic matter and nitrogen, enhances nutritional absorption and utilization, facilitating protein synthesis. This aligns with prior research; the use of manure, whether independently or combined with chemical fertilizer (50% cattle manure and 50% chemical fertilizer), markedly improved seed yield, protein content, and sunflower oil production (ESMAEILIAN et al., 2012). The elevated protein content by the application of potassium, organic N, and inorganic N enhanced the tolerance against drought stress by improving protein synthesis, stomatal regulation of the plant, as it was stated by CAKMAK (2005). This study indicates that K+GOIM treatment, which elevates moisture and protein levels, frequently leads to a reduction in oil content. Fertilizers that enhance protein content, particularly those abundant in nitrogen, drive the plant to focus on protein synthesis rather than oil accumulation. Moreover, elevated moisture content signifies greater water retention in the seed, which may disrupt the oil synthesis process, as oil accumulation typically occurs later in seed maturity when moisture levels diminish. This trade-off among protein, moisture, and oil content signifies a natural resource allocation, prioritizing protein and moisture retention at the expense of oil production.

5.2.4. Effect of fertilizer treatment on fatty acid composition of sunflower oil

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. Our result aligns with prior research indicating that during drought circumstances, the levels of palmitic and linoleic acids rise. Conversely, the concentrations of stearic and oleic acids often diminish (GHAFFARI et al., 2023b). Drought stress strongly affects the unsaturation ratio of oleic and linoleic acids in sunflower oil, altering the fatty acid composition, as evidenced by FLAGELLA et al. (2002). Research by FLAGELLA et al. (2000) indicates that throughout the interval from blooming to physiological maturity, plants experiencing water scarcity have elevated levels of oleic acid and reduced levels of linoleic acid compared to those that are sufficiently hydrated. Research by ALI et al. (2009) indicates

that drought stress diminishes linolenic acid levels while elevating stearic acid levels. Notwithstanding this alteration, the overall unsaturated fatty acid content remained constant in both drought-stressed and conventionally watered plants of both cultivars, however, saturated fatty acid levels rose in the first cultivar. In the same research, however, there was no alteration in palmitic acid levels in either cultivar. Our work corroborates the observation that palmitic acid remained unchanged under varying water conditions; however, it contradicts the conclusion that monounsaturated fatty acids declined while saturated and polyunsaturated fatty acids rose. The concentration of fatty acids in sunflower oil differs among treatments. 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. Our findings do not align with the research conducted by ALZAMEL et al. (2022b), which examined the impact of inorganic fertilizers, compost combined with biofertilizer, and filter mud cake on the quality of sunflower oil. According to the findings, organic fertilizers were essential for increasing seed and oil output, while inorganic fertilizers were more successful at promoting plant development and oil quality characteristics, including phenolic metabolites and chlorophyll content. The adverse effect of compost combined with biofertilizer on oleic acid content indicates that fertilizer selection might distinctly influence sunflower oil composition, highlighting the necessity for meticulous decision based on the intended crop yield. 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. Fatty acids enhance both the nutritional and commercial value of the oil (BABAR et al., 2024; LI et al., 2018). Additionally, by improving the biochemical characteristics and seed components, potassium fertilization helped sustain greater oil quality under drought stress conditions, guaranteeing that the sunflower seeds generated healthy, high-quality oil even in difficult circumstances (ZAMANI et al., 2020). 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. The desaturase genes that facilitate the conversion of oleic acid to linoleic and linolenic acid (fad2a and fad3 genes) are increased following the application of organic manure (MA et al., 2024). 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). A research study indicated that organic manure, especially sheep dung, enhances unsaturated fatty acids such as linoleic acid and linolenic acid in oilseed flax. Both investigations indicate that organic treatments augment the formation of advantageous fatty acids, essential for enhancing oil quality and nutritional value (MA et al., 2024). Treatments that enhanced linoleic acid frequently led to decreased oleic acid levels, and the reverse was also true. In the same vein, the article discovered that linolenic acid accumulation was negatively correlated with oleic acid and linoleic acid. This is because linolenic acid is synthesized from linoleic acid through desaturation (MA et al., 2024). 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 (MA et al., 2024). 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. Our discovery is consistent with other findings in sesame oil, where the effective microorganism may facilitate the biosynthesis of oil and the unsaturated activities that stimulate the synthesis of polyunsaturated fatty acids (SALAMA et al., 2015). 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

113

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 research is consistent with the findings of (SHOGHI-KALKHORAN et al., 2013), who reported that the highest concentrations of linoleic acid and oleic acid were observed in the 50% farmyard + 50% chemical and 100% chemical + farmyard treatments, respectively. 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. Some authors have reported that higher oleic acid content, but 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 (TEKAYA et al., 2013). 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.

5.2.5. Year-Specific Responses of Sunflower Yield Parameters to Fertilizer Treatments: Insights from Multivariate ANOVA, Heatmap, and AMMI Analysis

The multivariate ANOVA results offer a clear statistical indication that the K+GOIM treatment substantially increased several important yield parameters, including head weight, head diameter, TKW, seed weight, and total yield, particularly in 2022. These results emphasize the efficacy of this treatment in enhancing productivity and growth during that year. The potential of K+GOIM as a high-yield fertilizer for sunflower cultivation is supported by this initial discovery, which establishes it as a strong performer under the specific environmental conditions of 2022. The heatmap analysis further substantiates these findings by visually emphasizing the substantial positive effect of K+GOIM on specific characteristics, such as head weight and seed weight, in comparison to other treatments. K+GOIM consistently influences these yield parameters, as evidenced by the heatmap, which generalizes the multivariate ANOVA results across all years. The conclusion that K+GOIM is effective in enhancing yield-related traits is supported by this consistency, although the multivariate ANOVA indicated that these effects were most pronounced in 2022. The statistical results from the multivariate

ANOVA are further supported by the positive changes in head weight and seed weight observed in the heatmap, which confirm that these are critical characteristics that are influenced by the K+GOIM treatment. By explicating the variability in treatment responses from year to year, the AMMI analysis provides an additional insight. In 2022, the K+GOIM treatment resulted in the maximum head weight, as per AMMI. However, in 2023, the GOIM+EM treatment increased the seed weight and seed number per head. This variation in treatment efficacy between years can be ascribed to environmental factors (such as soil moisture, temperature, or weather conditions) that varied between 2022 and 2023. The interaction effects captured by AMMI demonstrate that the efficacy of treatment is context-dependent, with specific treatments performing optimally under specific environmental conditions. The results of the multivariate ANOVA, heatmap, and AMMI analyses collectively indicate that K+GOIM is an effective fertilizer treatment; however, its effectiveness is significantly dependent on favorable environmental conditions, such as those that were observed in 2022. The potential of K+GOIM to substantially increase sunflower productivity is suggested by the strong positive impact it had on head weight and seed weight in 2022. However, the AMMI PCA analysis also suggests that other treatments, such as GOIM+EM, might perform better than K+GOIM under varying conditions, as evidenced by the higher seed number per head and seed weight in 2023. This emphasizes the significance of taking environmental interactions into account when assessing the long-term efficacy of fertilizer applications. A treatment that is effective in one year may not necessarily offer the same benefits in subsequent years as a result of evolving environmental conditions. Consequently, these findings underscore the necessity of a fertilization strategy that is both adaptable and flexible. This strategy may involve the integration of treatments such as K+GOIM and GOIM+EM to optimize sunflower production across a variety of environmental conditions and years. The sunflower yield in 2022 was positively impacted by K+GOIM, while the yield in 2023 was positively impacted by GOIM+EM, as illustrated by field results. These results indicate that productivity can be improved through the use of integrated fertilizer treatments; however, their efficacy is contingent upon environmental variables, including soil type, water availability, and temperature. Our findings are more specific to the year 2022—a particularly dry year—and show greater significance for that year compared to others. This could be attributed to the lack of annual soil fertility assessments, which should ideally precede fertilization programs, as well as insufficient analysis of microbiological activity. 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.

5.2.6. Year-Specific Responses of Sunflower Fatty Acid Composition to Fertilizer Treatments: Insights from Multivariate ANOVA, Heatmap, and AMMI Analysis

A comprehensive visualization of the impact of specific fertilizers on individual fatty acids is provided by the heatmap. According to the heatmap results, EM had a substantial positive impact on the PUFA ω-3 and alpha-linolenic acid, both of which are essential for human health. Simultaneously, the GOIM+EM treatment exhibited a negative impact on linoleic acid and PUFA ω-3, indicating a differential response in the fatty acid composition when effective microorganisms are combined with organic and inorganic nutrients. The ANOVA results, which suggest that EM promotes higher PUFA levels, are consistent with the positive changes in PUFA ω-3 and alpha-linolenic acid under EM. Nevertheless, the heatmap's indication of the adverse impact of GOIM+EM on linoleic acid and PUFA ω-3 suggests that this combination may result in a decrease in specific PUFAs while promoting MUFAs, as also suggested by the multivariate ANOVA. The year-specific effects of fertilizer treatments are further clarified by the AMMI analysis. In 2022, both K+GOIM and EM increased PUFA ω-3 and alpha-linolenic acid, as indicated by AMMI, indicating a strong year-dependent response. This implies that these treatments were notably effective in producing higher levels of beneficial PUFAs in the environmental conditions of 2022. Nevertheless, the absence of substantial effects in 2023 and 2024 suggests that the efficacy of these fertilizers may be susceptible to environmental fluctuations, such as temperature or precipitation levels during the growing season. The AMMI analysis underscores that K+GOIM and EM were effective in increasing PUFA levels in 2022; however, these effects were not sustained across subsequent years, underscoring the impact of environmental factors on treatment efficacy. This year-specific response emphasizes the necessity of adaptive management strategies that take into account environmental conditions when selecting fertilizer treatments. The fatty acid composition, moisture content, and protein levels of sunflower seeds are influenced by fertilizer treatments in a complex and year-dependent manner, as indicated by the combined results of the multivariate ANOVA, heatmap, and AMMI analysis. The potential for these treatments to improve the nutritional content of sunflower oil, particularly in years with favorable growing conditions, is indicated by the positive effects of K+GOIM and EM on PUFA and alpha-linolenic acid in 2022. Nevertheless, the decrease in linoleic acid and PUFA ω-3 observed under GOIM+EM raises concerns about the long-term stability of these effects and implies that this

treatment may be more appropriate for increasing MUFAs than PUFAs. In conclusion, the results indicate that K+GOIM and EM are effective fertilizer treatments for increasing PUFA and alpha-linolenic acid, particularly in the presence of drought, such as the 2022 drought. However, the efficacy of these strategies 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. The contrasting effects of GOIM+EM on linoleic acid and PUFA ω-3 also suggest that a variety of treatments can have a complex impact on specific fatty acid profiles, emphasizing the importance of selecting a targeted treatment based on the desired oil characteristics.

6. CONCLUSION AND RECOMMENDATIONS

6.1.   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 resulted in an increase in 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, producing oil rich in 32% PUFA (high nutritional value omega-3 and 6) and 57% MUFA. Although its effectiveness was strongly influenced by environmental conditions.

6.2. Recommendations for the future

6.2.1. 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.

6.2.2. 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.

7. 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 + 50kg/ha of dried-pelleted cattle manure) enhanced yield traits. In contrast, the wetter season favored GOIM + EM (50 kg/ha of Ammonium nitrate + 50kg/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 + 50kg/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.

8. SUMMARY

Sunflowers are a valuable oilseed crop, but their productivity is often limited by inadequate germination conditions, poor seedling establishment, and ineffective fertilizer management. Factors such as temperature, water availability, seed number, and fungal growth significantly influence germination success and early growth stages, while fertilizer treatments can have varying effects on crop performance and productivity depending on environmental conditions. The research problem addressed in this study focuses on optimizing the germination conditions and integrating fertilizer strategies to enhance sunflower growth, yield, and oil quality, particularly in challenging environmental conditions such as drought. The germination of sunflower seeds was investigated under controlled conditions at eight consistent temperatures: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C. For the water test, there were 12 water levels based on one-milliliter intervals and 18 water levels based on TKW. In addition, four seed densities (6, 8, 10, and 12) and two antifungal application techniques (sterilization and growing medium) were examined. Seed germination rate, seedling length, and seedling dry matter were monitored during this experiment. The optimal temperature range for germination and early seedling growth was 15-30°C. At 25°C, the seedling grows optimally and reaches its maximum length. These results can be used in prediction of sowing time of sunflower seeds. TKW was found to be an interesting method to determine the water needed for sunflower seeds. Sunflower seeds can germinate under a wide range of water availability. The optimal range for seedling development (8.2–11.4) is wider than the optimal range for dry matter accumulation, which is 4.4–8.4 ml or 850–1750% of the TKW. The study highlighted that a density of 10 to 12 seeds per 9 cm Petri dish demonstrates the most exceptional values and is advantageous for future research and breeding projects, particularly when seeds are scarce. Seed priming is a more effective antifungal application technique than other techniques. These optimized germination and early growth conditions may support breeding programs with scarce seed availability and serve as a basis for microgreen production. The objective of the field experiment study was to examine the impact of organic fertilizers, inorganic fertilizers, and biofertilizers, as well as their combinations, on the growth, yield, and oil quality of sunflowers in rainfed conditions. Additionally, the study sought to determine the effectiveness of integrated fertilizer treatment in optimizing sunflower productivity over three years. In comparison to the control, we have six treatments for this objective: potassium K, 50% organic nitrogen, 50% inorganic nitrogen GOIM, effective microorganism EM, K+GOIM, GOIM+EM, and K+EM. The treatments were applied at the vegetative stage (BBCH 14). Growth parameters (plant

height, leaf number, stem diameter), physiology parameters (LAI, SPAD output, fluorescence), yield parameters (head diameter, head weight, seed weight, seed number per head, TKW, yield), seed quality (protein content, moisture content, oil content), and fatty acid composition. The year 2022 is the driest of the experimental years, characterized by high temperatures and minimal precipitation. In 2022, the fertilizer treatment's interaction was more significant. The growth parameters (leaf number and stem diameter), physiology parameters (LAI, SPAD output), protein and moisture content of the seeds, and unsaturated fatty acids (linoleic and alpha-linolenic acid; PUFA) were all enhanced by the combination of organic nitrogen, inorganic nitrogen, and potassium (K+GOIM). The EM is the second most effective treatment after K+GOIM, as it improved the chlorophyll content (SPAD output), leaf number, moisture content, and linoleic and alpha-linolenic PUFA. Additionally, the potassium K treatment demonstrated interesting results in terms of stem diameter, yield, oil content, oleic acid, and eicosenoic acid MUFA in this study. The GOIM+EM also demonstrated interesting results in 2022, specifically in the improvement of oleic acid and the enhancement of yield attributes in 2023. The heatmap and AMMI PCA, when combined, corroborated the multivariate two-way ANOVA, which demonstrated that K+GOIM demonstrated consistent results in head weight and seed weight, as well as polyunsaturated acid, which was followed by EM. Nevertheless, the polyunsaturated acid was substantially reduced by GOIM+EM, while the monounsaturated acid was increased (Figure 34). Overall, the K+GOIM fertilizer (90 kg potassium + 100 kg nitrogen; 50% organic, 50% inorganic) emerged as the most effective drought-resilient strategy, significantly enhancing sunflower physiology and yield (+43%). It also promoted oil quality, rich in 32% PUFA (omega-3 and 6, indicating high nutritional value) and 57% MUFA. Its effectiveness was most pronounced under drought conditions similar to those observed in 2022. Together, the findings of germination experiment and fertilizers management provide a robust, empirically validated framework for optimizing sunflower production through precision germination protocols and climate-smart fertilization strategies, with particular relevance for drought-prone agricultural systems.

Summary of Multivariate Analyses and Treatment Effects on Sunflower Traits on Oil Quality

Multivariate two-way ANOVA | Heatmap clustering | AMMI PCA

K+GOIM
+ Physiological traits
+ Yield attributes
↑ PUFA
↓ MUFA

EM
+ Physiological traits
↑ PUFA

GOIM+EM
↑ MUFA
↓ PUFA
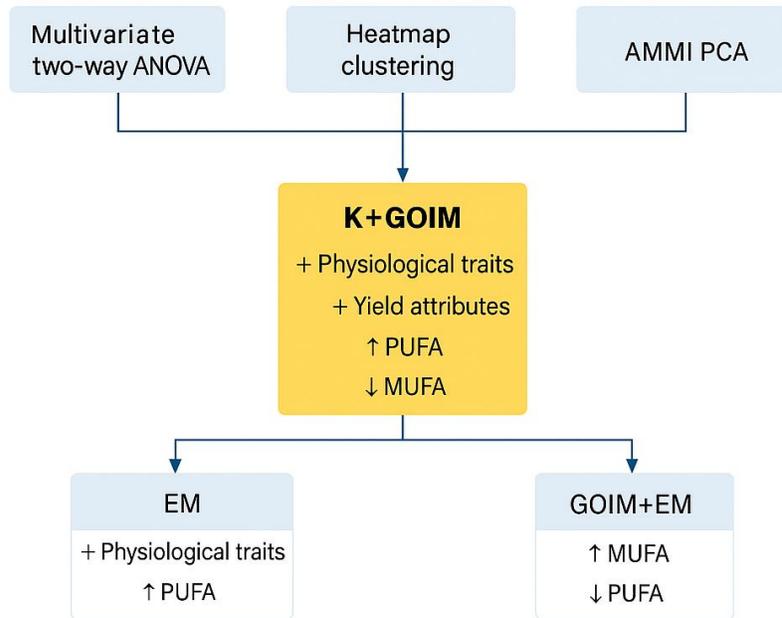
GOIM = Combined Organic and Inorrganic Manure; EM = Effective Microorganism
PUFA = Polyunsaturated Fatty Acids, MUFA = Monounsaturated Fatty Acida

Figure 34.Fertilizers strategy effects on Fatty acid composition.

APPENDIXES

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Statistical tables

Table B. 1.Post hoc Results of Fertilizer Effects on seed quality

Treatment	Year	Moisture_content	Raw_protein	Oil_content
CONTROL	2022	3.9±0.1Bb	13.267±0.252Bb	47.9±0.1Aab
	2023	5.767±0.153Aa	14.457±0.475Bab	41.257±2.048Ab
	2024	4.2±0.173Ba	16.9±0.624Aa	42.767±3.099Aa
EM-1	2022	4.833±0.076Aa	15.21±1.282ABab	42.873±1.241Ad
	2023	5.567±0.252Aa	14.147±0.795Babc	42.07±1.146Aab
	2024	4.1±0.173Ba	17.467±0.981Aa	43.667±1.436Aa
GOIM	2022	4±0Bb	15.7±0.265Ba	46.733±0.643Abc
	2023	5.833±0.115Aa	12.777±0.545Cbc	40.72±1.53Bb
	2024	4.367±0.115Ba	18.133±0.764Aa	41.567±2.268ABa
GOIM+EM-1	2022	4.067±0.058Bb	16.467±0.058Aa	45.7±0.52Ac
	2023	5.467±0.115Aa	12.77±0.449Bbc	43.007±0.988Cab
	2024	4.333±0.153Ba	17.4±0.954Aa	43.167±1.35ABa
K	2022	3.967±0.058Bb	12.667±0.058Ab	48.7±0.361Aa
	2023	5.867±0.306Aa	15.41±1.34Aa	40.64±2.472Ab
	2024	4.2±0.173Ba	15.8±1.609Aa	43.867±2.214Aa
K+EM-1	2022	4±0Bb	12.967±0.153Bb	48±0.436Aab
	2023	5.767±0.416Aa	12.287±0.081Cc	40.517±3.083ABb
	2024	4.2±0.1Ba	17.333±0.643Aa	43.7±0.985Ba
K+GOIM	2022	4.883±0.076Aa	15.303±0.442Ba	42.823±0.499Ad
	2023	4.267±0.208Bb	13.55±0.795Babc	47.367±1.779Aa
	2024	4.3±0.1Ba	17.833±0.451Aa	42.633±0.058Aa

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. The star symbol denotes the statistical interaction between two factors (Year*Fertilizers treatment).

Table B. 2.Post hoc Results of Fertilizer Effects on growth and physiological parameters of sunflower

Treatment	Year	LAI	leaf number	Plant height (cm)	Spad output	Fluoresnce	stem_diameter (cm)
CONTROL	2022	0.6±0.2Cb	6.6±1.2Cc	188.9±2.7Aa	26.5±3.6Bb	0.8±0.01Aa	16.5±2.4Bab
	2023	3.5±0.2Aa	23.4±1Aa	186±5.4Aa	35.367±4.13Aa	0.8±0.03Aa	31.5±0.7Aa
	2024	2±0.3Ba	16±0.6Ba	131±9.9Ba	35.5±2Aa	0.8±0.01Aa	17±2Ba
EM-1	2022	1.1±0.7Bb	11.6±2.5Bac	175.2±10Aab	33.8±2.9Aab	0.8±0.01Aa	13.6±2.8Bb
	2023	3.8±0.2Aa	22.6±0.9Aa	180±7Aa	35.5±2Aa	0.8±0.04Aa	32±2.6Aa
	2024	1.8±0.4Ba	15±1.2Ba	114±8.7Ba	35.6±0.7Aa	0.8±0.02Aa	17±5Ba
GOIM	2022	1.1±0.1Bb	9.9±1.4Cc	175.±4.6Aab	26.5±1Bb	0.8±0.02Aa	14.9±5Bab
	2023	3.2±0.791Aa	21.8±1.4Aa	175.6±18.8Aa	35.4±0.7Aa	0.8±0.04Aa	27.9±5.5Aa
	2024	2.±0.2ABa	16.6±0.5Ba	111.7±36.5Ba	34.3±2Aa	0.8±0.02Aa	19.1±1.4ABa
GOIM+EM-1	2022	0.6±0.3Cb	8.6±3.08Bbc	140±3.2Bc	29.2±2.1Bab	0.8±0.02Aa	11.4±1.02Cb
	2023	3.3±0.7Aa	23.1±0.4Aa	179±7.6Aa	35.7±3.4Aa	0.8±0.02Aa	33.6±1.9Aa
	2024	2.2±0.2Ba	16.4±0.9Ba	124±22.6Ba	35.3±0.9Aa	0.8±0.04Aa	17.8±3.02Ba
K	2022	1±0.09Bb	7±3Bbc	175.5±2.6Aab	26.9±3.9Bb	0.8±0.01Aa	16±3.6Bab

Treatment	Year						
	2023	3.3±0.3Aa	22.6±0.4Aa	172.6±14Aa	36.6±2.7Aa	0.8±0.01Aa	30.5±2.8Aa
	2024	1.8±0.5Ba	15.5±2.1Ba	139±29.8Aa	34.2±2.2ABa	0.8±0.02Aa	20.5±2.6Ba
K+EM-1	2022	0.6±0.4Bb	8.4±0.2Cc	155.7±9.5ABbc	26.5±3.08Bb	0.8±0.02Aa	12.8±4.4Bb
K+EM-1	2023	3.1±0.8Aa	23±0.5Aa	173±17Aa	34.3±1.4Aa	0.8±0.05Aa	32.8±4.5Aa
K+EM-1	2024	2.1±0.2Aa	16.5±0.5Ba	134.5±16.3Ba	33.7±1.6Aa	0.8±0.04Aa	20.8±0.8Ba
K+GOIM	2022	2.9±0.5Aa	19±0.4Bab	163.7±20.3Aabc	35.4±1.1Aab	0.8±0.02Aa	23.6±2.1Aa
K+GOIM	2023	3.42±0.5Aa	22±0.9Aa	179±27.6Aa	35.1±1.3Aa	0.8±0.02Aa	26.6±3.6Aa
K+GOIM	2024	2.3±0.6Aa	16±0.5Ca	148±17Aa	35.6±0.9Aa	0.8±0.02Aa	20.7±2.9Aa

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. The star symbol denotes the statistical interaction between two factors (Year*Fertilizers treatment).

Table B. 3.Post hoc Results of Fertilizer Effects on Yield attributes

Treatment	Year	head_diameter (cm)	head weight (g)	seedweight (g)	SN/head	TKW (g)	Yield (t/ha)
CONTROL	2022	8.24±0.36Bb	10.72±1.84Bb	121.347±4.155Cc	1434.4±147 Aa	29.7±0.8Cb	1.703±0.142Ba
	2023	17.83±1.70Aab	161±36.6Aa	988.7±165.8Aa	1773.9±117.7Aa	52.8±3.2Bab	3.737±0.04Aa
	2024	13.49±3.13ABa	79±28.8ABa	410±37Bb	1094.9±375Aa	42.9±3.4Aa	1.9±0.772ABa
EM-1	2022	6.90±0.80Bbc	16.3±4Ab	17.5±2.9Bd	1505±313. Aa	29.0±3.8Ab	1.723±0.28Aa
	2023	17.33±2.80Aab	179.5±76.9Aa	1066±307.8Aa	1926.6±216Aa	59.6±11.9Aab	4.657±1.379Aa
	2024	12.25±2.80ABa	87.5±33.3Aa	496.7±268ABb	1416.8±156.6Aa	41.1±8.5Aa	2.313±0.44Aa
GOIM	2022	8.32±0.97Bbc	12±5Bb	198±66Cbcd	1421.6±80.3Aa	37.4±2.8Bb	2.12±0.159Ba
	2023	15.15±0.57Ab	141±44ABa	751.6±57.5Aa	1549.6±119Aa	46.3±0.660Ab	2.867±0.24Aa
	2024	15.57±0.68Aa	96.9±10.9Ba	552±22Ba	1293.5±97.4Aa	48.7±4.86ABa	2.507±0.078ABa
GOIM+EM-1	2022	5.5±1.35Cbc	8±2.7Cb	171.5±16.6Bbc	1603±67.9Aa	28.4±1.1Cb	1.817±0.023Ba
	2023	19±0.87Aa	200.9±33.8Aa	1180.3±246.8Aa	1935.6±177.9Aa	63.2±2.6Aa	4.893±0.519Aa
	2024	14.04±0.45Ba	89.±18.7Ba	546.2±94.9Ab	1270.7±51Aa	46.5±5.6Ba	2.367±0.332Ba
K	2022	7.55±0.31Bbc	11±5.8Bb	191±6Ab	1678±64.5Aa	29.8±1.2Bb	2±0.151Ba
	2023	18.17±1.88Aab	175±44Aa	1054.3±256Aa	1979.7±271.3Aa	50.78±2.2Ab	4.027±0.621Aa
	2024	14.77±3.05ABa	97±25Aa	517±120.2Ab	1376.5±166.9Aa	42.3±4.2ABa	2.33±0.417Ba

Treatment	Year						
K+EM-1	2022	6.79±0.26Cc	8.6±4Ab	161.8±12Abc	1425.8±201.8Aa	27.7±1.7Bb	1.573±0.127Aa
	2023	18.3±2.48Aab	154.6±54.7Aa	1017±500.6Aa	1886.9±494.9Aa	47.3±4.7Aab	3.58±1.072Aa
	2024	13.77±1.338Ba	100±30.8Aa	468.8±190.8Ab	964.6±165.4Aa	50.3±6.4Aa	1.967±0.577Aa
K+GOIM	2022	14.56±0.59ABa	99.5±2.5Ba	672±47Ba	1496.6±152.5Aa	49.3±2.3Aa	2.96±0.469Aa
	2023	16.07±0.333Aab	123±3Aa	820±40.8Aa	1693.7±59.23Aa	52.3±1.5Ab	3.543±0.19Aa
	2024	13.057±0.904Ba	76.023±5.738Ca	524±55.83Bb	1299.49±345.336Aa	46.233±7.396Aa	2.457±1.025Aa

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. The star symbol denotes the statistical interaction between two factors (Year*Fertilizers treatment)

159

Table B. 4.Post hoc Results of Fertilizer Effects on fatty acid composition of sunflower crop

Treatment	Year	Myristic_acid	Palmitic_acid	Palmitoleic_acid	Margaric_acid	Stearic_acid	Oleic_acid	Linoleic_acid	Alpha_linolic_acid	Arachidic_acid	Eicosenoic_acid	Behenic_acid	Tricosanoic_acid	Lignoceric_acid
CONTROL	2022	0.03±0B*a	4.7±0.3A*a	0.13±0A*a	0.03±0A*a	2.8±0.2B*ab	70.9±0.1B*bc	19.3±0.3B*bc	0.03±0B*b	0.3±0.005AB*b	0.3±0A*bc	1.1±0.07B*a	0.05±0.003A*ab	0.4±0.02B*bc
	2023	0.04±0.006A*a	5.2±0.3A*a	0.05±0.02B*a	0.04±0.01A*a	3.6±0.3A*a	28.5±3.7C*a	60.3±3.8A*a	0.06±0.005A*a	0.3±0.03B*a	0.14±0.01A*a	1±0.1B*a	0.04±0.003A*a	0.3±0.04C*a
	2024	0.03±0.006B*a	3.9±0.15B*a	0.1±0.015Aa	0.03±0.006A*a	2.8±0.2B*a	84±2.7A*ab	6.3±2.4C*a	0.03±0B*a	0.4±0.02A*a	0.3±0.006A*a	1.3±0.05A*a	0.05±0.006A*a	0.5±0.02A*a
EM-1	2022	0.033±0.003AB*a	4.588±0.296A*a	0.092±0.003B*b	0.032±0.006A*a	3.227±0.236AB*ab	57.84±2.667B*cd	32.075±2.735B*ab	0.043±0.006A*a	0.31±0.022AB*b	0.207±0.003B*d	1.118±0.077B*a	0.04±0*c	0.37±0.018B*c
	2023	0.043±0.006A*a	5.477±0.573AB*a	0.05±0.01C*a	0.037±0.006A*a	3.57±0.42A*a	30.91±0.96C*a	58.09±0.89A*a	0.05±0A*a	0.273±0.029B*a	0.14±0C*a	0.92±0.095C*a	0.03±0*a	0.26±0.01C*a
	2024	0.023±0.006B*a	3.7±0.07B*a	0.133±0.006A*a	0.027±0.006A*a	2.753±0.031B*a	86.53±0.66A*a	3.627±1.342C*a	0.03±0B*a	0.347±0.015A*a	0.273±0.006A*a	1.317±0.059A*a	0.05±0*a	0.48±0.026A*a
GOIM	2022	0.03±0B*a	4.25±0.036B*a	0.113±0.006B*ab	0.04±0A*a	2.94±0.026B*a	73.05±0.182B*a	17.29±0.262B*d	0.03±0B*b	0.337±0.006AB*ab	0.26±00bc	1.18±0.06B*a	0.05±0*a	0.423±0.025A*abc
	2023	0.04±0A*a	5.28±0.147A*a	0.05±0C*a	0.043±0.006A*a	4.03±0.171A*a	30.397±1.438C*a	58.317±1.25A*a	0.055±0.005A*a	0.31±0.017B*a	0.15±00a	1.007±0.067B*a	0.04±0*a	0.283±0.025B*a
	2024	0.023±0.006B*a	3.8±0.01C*a	0.137±0.006A*a	0.03±0B*a	3.013±0.188B*a	85.2±1.389A*ab	5.167±1.677C*a	0.03±0B*a	0.377±0.031A*a	0.26±00a	1.38±0.104A*a	0.05±0*a	0.497±0.038A*a

GOIM+EM-1	2022	0.03±0A*a	3.983±0.119B*a	0.113±0.006B*ab	0.04±0A*a	3.0733±0.085AB*ac	79.653±1.758B*ab	10.683±1.38B*e	0.04±0B*ab	0.367±0.025A*a	0.267±0.006A*b	1.237±0.105A*a	0.053±0.006A*a	0.45±0.04A*ab
	2023	0.04±0.01A*a	5.417±0.477A*a	0.05±0.01C*a	0.037±0.006AB*a	3.775±0.605A*a	29.02±3.222C*a	59.93±3.402A*a	0.060.01A*a	0.283±0.032B*a	0.14±0B*a	0.94±0.061B*a	0.033±0.006B*a	0.263±0.023B*a
	2024	0.023±0.006A*a	3.753±0.064B*a	0.137±0.006A*a	0.027±0.006B*a	2.723±0.178B*a	86.243±1.563A*a	4.67±1.333C*a	0.03±0B*a	0.337±0.023AB*a	0.27±0.01A*a	1.263±0.038A*a	0.05±0A*a	0.463±0.021A*a
K	2022	0.03±0B*a	4.19±0.046B*a	0.123±0.006A*a	0.03±0A*a	2.71±0.05B*b	76.25±0.885B*a	14.29±1.042B*de	0.037±0.006B*ab	0.33±0.01A*ab	0.28±0A*a	1.213±0.059A*a	0.05±0A*a	0.477±0.038A*a
	2023	0.043±0.006A*3	5.453±0.493A*a	0.05±0.01B*a	0.04±0.01A*a	4.32±0.21A*a	28.21±3.575C*a	60.27±3.543A*a	0.06±0A*a	0.313±0.023A*a	0.14±0B*a	1.003±0.055B*a	0.036±0.006B*a	0.283±0.021B*a
	2024	0.027±0.006B*3	3.87±0.147B*a	0.15±0.017A*a	0.023±0.006A*a	2.63±0.193B*a	85.987±1.392A*ab	4.84±1.569C*a	0.03±0B*a	0.33±0.017A*a	0.273±0.012A*a	1.263±0.059A*a	0.043±0.006AB*a	0.47±0.02A*a
K+EM-1	2022	0.03±0B*a	4.53±0.346AB*a	0.137±0.012A*ab	0.03±0B*a	2.817±0.025B*ac	73.95±1.09A*ab	16.347±0.897B*cd	0.04±0B*ab	0.32±0.01A*b	0.255±0.005A*c	1.083±0.042AB*a	0.04±0A*c	0.413±0.006A*abc
	2023	0.043±0.006A*a	5.727±0.585A*a	0.057±0.012B*a	0.04±0A*a	3.757±0.295A*a	28.36±4.233B*a	60.1967±3.966A*a	0.055±0.005A*a	0.303±0.015A*a	0.14±0A*a	0.977±0.042B*a	0.03±0.01A*a	0.3±0.026B*a
	2024	0.023±0.006B*a	4.003±0.481B*a	0.137±0.015A*a	0.027±0.006B*a	2.843±0.289B*a	77.485±7.125A*b	4.15±1.83C*a	0.03±0C*a	0.33±0.02A*a	0.178±0.15A*a	1.207±0.107A*a	0.045±0.005A*a	0.427±0.04A*a
K+GOIM	2022	0.03±0.005B*a	4.555±0.203B*a	0.093±0.003B*c	0.037±0.008AB*a	3.287±0.308B*a	57.89±1.137B*d	31.978±1.28B*a	0.045±0.005B*a	0.318±0.019B*b	0.208±0.003B*d	1.137±0.031B*a	0.042±0.003AB*bc	0.377±0.013B*c
	2023	0.04±0A*a	5.337±0.242A*a	0.05±0C*a	0.047±0.006A*a	4.033±0.155A*a	29.4±1.532C*a	59.297±1.415A*a	0.06±0A*a	0.303±0.006B*a	0.143±0.006C*a	0.987±0.015C*a	0.035±0.005B*a	0.277±0.015C*a

| | 2024 | 0.02±0C*a | 3.693±0.076C*a | 0.137±0.006A*a | 0.027±0.006B*a | 2.797±0.042B*a | 86.76±1.075A*a | 4.027±0.974C*a | 0.03±0C*a | 0.353±0.006A*a | 0.277±0.006A*a | 1.333±0.025A*a | 0.05±0A*a | 0.49±0.01A*a |

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. The star symbol denotes the statistical interaction between two factors (Year*Fertilizers treatment).

Table B. 5.Multivariate Two-Way ANOVA Results of Fertilizer Effects on growth and Physiological parameters

				Multivariate Tests		

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment	Wilks' Lambda	0.16	2.32	36.00	165.24	<0.001
Year	Wilks' Lambda	0.01	64.11[b]	12.00	74.00	<0.001
Treatment*Year	Wilks' Lambda	0.06	2.03	72.00	207.11	<0.001

			Tests of Between-Subjects Effects			

Dependent variables	source of variation	Type III Sum of Squares	df	Mean Square	F	Sig.
LAI	Treatment	5.54	6	0.92	4.78	0.00
	Year	54.03	2	27.01	139.86	<0.001

	treatment*year	7.83	12	0.65	3.38	0.00
leaf number	Treatment	89.19	6	14.87	7.32	<0.001
	Year	1634.20	2	817.10	402.28	<0.001
	treatment*year	245.91	12	20.49	10.09	<0.001
Plant height (cm)	Treatment	2710.02	6	451.67	1.62	0.17
	Year	28039.52	2	14019.76	50.32	<0.001
	treatment*year	5495.89	12	457.99	1.64	0.116
Spad output	Treatment	116.71	6	19.45	3.55	<0.001
	Year	491.83	2	245.92	44.92	<0.001
	treatment*year	162.64	12	13.55	2.48	0.02
Fluoresnce	Treatment	0.01	6	0.00	3.33	0.01
	Year	0.02	2	0.01	12.68	<0.001
	treatment*year	0.00	12	0.00	0.42	0.95
stem_diameter (cm)	Treatment	59.99	6	10.00	0.96	0.46

Year	2649.61	2	1324.81	127.09	<0.001
treatment*year	401.69	12	33.47	3.21	0.00

Table B. 6. Multivariate Two-Way ANOVA Results of Fertilizer Effects on yield attributes of sunflower crop

Multivariate Tests						
Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment	Wilks' Lambda	0.23	1.80	36	165.24	0.01
Year	Wilks' Lambda	0.03	30.19[b]	12	74	<0.001
Treatment * Year	Wilks' Lambda	0.05	2.07	72	207.11	<0.0010
Tests of Between-Subjects Effects						
Dependant variables	Source of varaiation	Type III Sum of Squares	Df	Mean Square	F	Sig.
head_diameter (cm)	Treatment	29.10	6	4.85	1.82	0.12
	Year	891.67	2	445.85	167.68	<0.001
	Treatment*Year	180.83	12	15.07	5.67	<0.001
head_weight (g)	Treatment	2551.27	6	425.21	0.46	0.83

	Year	201519.20	2	100759.60	108.79	<0.001
	Treatment*Year	31199.15	12	2599.93	2.80	0.01
Seed_weight (g)	Treatment	6257718.11	2	3128859.05	101.40	<0.001
	Year	231620.66	6	38603.44	1.25	0.30
	Treatment*Year	995433.85	12	82952.82	2.69	0.01
Seed_number	Treatment	606623.31	6	101103.88	2.169	0.07
	Year	3487098.44	2	1743549.22	37.40	<0.001
	Treatment*Year	466000.58	12	38833.38	0.83	0.62
TKW (g)	Treatment	461.64	6	76.94	3.40	0.008
	Year	4328.23	2	2164.11	95.52	<0.001
	Treatment*Year	1550.46	12	129.21	5.70	<0.001
Yield (T/ha)	Treatment	4.00	6	0.67	2.11	0.07
	Year	44.99	2	22.50	71.06	<0.001

	Treatment*Year	9.64	12	0.80	2.54	0.01

Table B. 7.Multivariate Two-Way ANOVA Results of Fertilizer Effects on seed quality of sunflower crop

Multivariate Tests						
Effect		Value	F	Hypothesis df	Error df	Sig.
Treatment	Wilks' Lambda	0.35	2.84	18.0	113.62	<0.001
Year	Wilks' Lambda	0.01	117.04	6.0	80.00	<0.001
Treatment *Year	Wilks' Lambda	0.03	7.41	36.0	118.91	<0.001
Tests of Between-Subjects Effects						
Dependent Variables	Source of variation	Type III Sum of Squares	df	Mean Square	F	Sig.
Moisture_content	Treatment	0.63	6	0.11	3.68	<0.01
	Year	22.42	2	11.21	393.45	<0.001
	Treatment*Year	8.57	12	0.72	25.09	<0.001
Raw_protein	Treatment	17.51	6	2.92	5.17	<0.001

	Year	151.28	2	75.64	134.07	<0.001
	Treatment*Year	56.01	12	4.67	8.27	<0.001
Oil_content	Treatment	19.90	6	3.32	1.26	0.29
	Year	175.32	2	87.66	33.52	<0.001
	Treatment*Year	204.99	12	17.08	6.53	<0.001

Table B. 8.Multivariate Two-Way ANOVA Results of Fertilizer Effects on fatty acid composition of sunflower crop

Multivariate Tests						
Effect		Value	F	Hypothesis df	Error df	Sig.
Wilks' Lambda	Treatment	0.00	2.72	108.00	150.45	<0.001
	Year	0.00	117.59[b]	36.00	50.00	<0.001
	Treatment * Year	0.00	2.09	216.00	279.46	<0.001
Tests of Between-Subjects Effects						

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Myristic_acid	Treatment	<0.001	6	<0.001	0.74	0.62
	Year	0.00	2	0.00	79.14	<0.001
	Treatment *Year	<0.001	12	<0.001	0.31	0.98
Palmitic_acid	Treatment	0.78	6	0.130	1.42	0.23
	Year	27.67	2	13.84	151.13	<0.001
	Treatment *Year	1.15	12	0.10	1.05	0.43
Palmitoleic_acid	Treatment	0.00	6	0.00	5.80	<0.001
	Year	0.09	2	0.04	496.00	<0.001
	Treatment *Year	0.00	12	0.00	2.93	0.00
Margaric_acid	Treatment	0.00	6	<0.001	2.08	0.08

	Year	0.00	2	0.00	33.69	<0.001
	Treatment *Year	0.00	12	<0.001	0.90	0.55
Stearic_acid	Treatment	0.61	6	0.10	1.73	0.14
	Year	13.64	2	6.82	115.22	<0.001
	Treatment *Year	1.92	12	0.16	2.70	0.00
Oleic_acid	Treatment	374.93	6	62.50	9.44	<0.001
	Year	34565.04	2	17282.52	2611.33	<0.001
	Treatment *Year	1194.70	12	99.56	15.04	<0.001
Linoleic_acid	Treatment	344.53	6	57.42	13.28	<0.001
	Year	33485.20	2	16742.60	3872.61	<0.001
	Treatment *Year	973.89	12	81.16	18.77	<0.001
α-Linolenic_acid	Treatment	0.00	6	<0.001	4.29	0.00
	Year	0.00	2	0.004	304.59	<0.001
	Treatment *Year	0.00	12	<0.001	3.78	0.00
Arachidic_acid	Treatment	0.00	6	0.00	2.30	0.05
	Year	0.03	2	0.01	33.12	<0.001

	Treatment *Year	0.01	12	0.00	2.1	0.04
Eicosenoic_acid	Treatment	0.01	6	0.00	1.66	0.16
	Year	0.18	2	0.09	79.35	<0.001
	Treatment *Year	0.03	12	0.00	2.07	0.04
Behenic_acid	Treatment	0.06	6	0.01	2.19	0.06
	Year	1.13	2	0.56	120.92	<0.001
	Treatment *Year	0.09	12	0.00	1.6	0.13
Tricosanoic_acid	Treatment	0.00	6	<0.001	4.91	0.00
	Year	0.00	2	0.00	65.57	<0.001
	Treatment *Year	0.00	12	<0.001	2.48	0.02
Lignoceric_acid	Treatment	0.01	6	0.00	2.53	0.03
	Year	0.42	2	0.21	324.76	<0.001
	Treatment *Year	0.03	12	0.00	3.72	0.00
SFA	Treatment	0.64	6	0.11	1.56	0.18
	Year	54.05	2	27.03	395.30	<0.001
	Treatment *Year	2.25	12	0.19	2.75	0.00

MUFA	Treatment	367.52	6	61.25	13.66	<0.001
	Year	36284.52	2	18142.26	4046.41	<0.001
	Treatment *Year	1040.01	12	86.67	19.33	<0.001
PUFA	Treatment	346.60	6	57.77	13.35	<0.001
	Year	33474.91	2	16737.45	3866.77	<0.001
	Treatment *Year	973.56	12	81.13	18.74	<0.001
PUFA_W6	Treatment	345.63	6	57.61	13.32	<0.001
	Year	33469.50	2	16734.75	3870.23	<0.001
	Treatment *Year	971.14	12	80.93	18.72	<0.001
PUFA_W3	Treatment	0.00	6	0.00	3.04	0.02
	Year	0.00	2	0.00	11.97	<0.001
	Treatment *Year	0.00	12	0.00	2.26	0.03

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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.