agriculture

MDPI

Article

Germination and Seedling Development Responses of Sunflower (*Helianthus annuus* L.) Seeds to Temperature and Different Levels of Water Availability

Asma Haj Sghaier [1], Hussein Khaeim [1,2], Ákos Tarnawa [1], Gergő Péter Kovács [1], Csaba Gyuricza [1] and Zoltán Kende [1,*]

[1] Institute of Agronomy, Hungarian University of Agriculture and Life Sciences, 2100 Gödöllő, Hungary
[2] Field Crops Department, College of Agriculture, University of Al-Qadisiyah, Al Diwaniyah 58002, Iraq
* Correspondence: kende.zoltan@uni-mate.hu

Abstract: Abiotic variables are crucial for seed germination and seedling development. In the present work, we attempted to determine the optimal conditions (temperature, water, seed density, and fungal growth) for sunflower seed development (*Helianthus annus* L. Larissza). 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 thousand kernel weight (TKW). In addition, four seed densities (6, 8, 10, and 12) and two antifungal application techniques (sterilization and growing medium) were examined. The results showed that temperature has a significant effect on seed germination, germination timing, and seedling development. Temperatures between 15 and 35 degrees Celsius were optimal for germination, with 25 degrees Celsius being the optimal temperature for significant germination and seedling development. Beginning at 0.6 mL, or 125% of the TKW, 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 5.8–8.2 mL or 1000–1625% of the TKW. The finding that a density of 10 to 12 seeds per 9 cm Petri dish demonstrates the most exceptional values 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.

Keywords: sunflower; seed germination; seedling development; seed density; temperature; water stress

Citation: Haj Sghaier, A.; Khaeim, H.; Tarnawa, Á.; Kovács, G.P.; Gyuricza, C.; Kende, Z. Germination and Seedling Development Responses of Sunflower (*Helianthus annuus* L.) Seeds to Temperature and Different Levels of Water Availability. *Agriculture* **2023**, *13*, 608. https://doi.org/10.3390/agriculture13030608

Academic Editor: Bernard Hauser

Received: 30 January 2023
Revised: 13 February 2023
Accepted: 21 February 2023
Published: 1 March 2023

1. Introduction

The establishment of a crop consists of three sub-phases: sowing through seed germination, seed germination through seedling emergence, and seedling emergence through first competition among young plants [1]. Seed germination is a three-step process. During the imbibition stage, dormant seeds absorb water and undergo hydrolysis. Variations in the amounts of proteins, carbohydrates, and lipids can influence the imbibition process, which is formed by the interaction of these three substances. Protein and oil bodies are the principal reserves in oilseed crops that provide energy, carbon, and nitrogen to the seedlings during germination [2]. The second step of germination regulation involves activating ATP production in glycolysis, the Krebs cycle, the respiratory chain, and the translation of stored mRNA. Germination is complete when the radicle emerges from the seed coat and produces a root, and the plumules construct a shoot system capable of absorbing inorganic substances, water, and light energy for healthy growth. The germination process impacts seedling survival rates and crop establishment, which climate influences. Inadequate seed germination can result in inadequate stand establishment, negatively impacting crop yield

and quality [3]. Furthermore, efficient seed germination affects seedling density per unit area [4,5]. Temperature and moisture are particularly key germination regulators [3].

Temperature plays a crucial role in germination processes, as it regulates membrane permeability and enzyme activity [4], germination, and the metabolic reaction of the seed [5]. The optimal temperature for maximizing germination and emergence varies depending on the crop [6,7]. Understanding crop seeds' germination and emergence response to temperature is crucial because it identifies their tolerance to low and high temperatures and the climatic conditions under which partial crops can germinate and establish successfully [7]. The high temperature may inhibit seed germination by decreasing the availability of energy and hydrolysates, thereby delaying or inhibiting the synthesis and activity of hydrolytic enzymes [8], including fatty acid oxidation, the breakdown of storage oil, and gluconeogenesis. Similarly, lower temperatures contribute to disruptions and delays in reserve mobilization [9,10], leading to seed viability loss and poor seedling development.

Water is essential for the germination of seeds. It is a medium for enzymatic processes, the solubilization and transport of metabolites, and a reagent in the hydrolysis of proteins, lipids, and carbohydrates in the storage tissues of germinating seeds [11]. A sufficient amount of soil moisture, or imbibition of the seed, is required for germination [12]. The soil moisture deficit at this stage inhibits the seed's ability to absorb the required water and delays germination by inhibiting the enzymes responsible for hydrolyzing endosperm starch into metabolizable sugars and providing energy for plant growth [11]. As a result, it might significantly reduce actual germination [13], resulting in poor, irregular, and non-uniform germination and crop stand [14]. In addition, drought stress can induce oxidative stress and increase the production of reactive oxygen species in seeds [15–18]. It causes a reduction in cell volume, which causes the cellular content to become more viscous, ultimately leading to the denaturation and aggregation of proteins and abnormal functioning of photosynthesis-related enzymes [19].

Sunflower (*Helianthus annuus* L.) is an annual oilseed crop belonging to the Asteraceae family. It is cultivated on 27.87 million hectares and produces 50.22 million metric tons, giving it an 8% share of the global oilseed market [20]. After soybean, oil palm, and canola crops, oilseed rape is the world's fourth most profitable and cost-effective crop [21]. Sunflower is a temperate zone crop that thrives in various soil and climate conditions because it is a rustic crop [22]. It can withstand an early autumn frost, which generally kills maize and soybeans [23]. In general, sunflowers are grown primarily for their seeds, which contain 40–50% oil and 17–20% protein, and thus can alleviate the gap between the production and consumption of edible oil and animal feed around the world. In addition, this oil crop has numerous health and nutrition benefits for heart patients. It protects against hypertension and cardiovascular disease because it contains relatively low cholesterol and a high proportion (90%) of unsaturated fatty acids [24,25]. Despite all these benefits, its production is threatened by various biotic and abiotic stressors, including drought and heat from germination to the filling stage and pests [26]. During germination, seeds are subject to various biotic and abiotic stresses that, individually or in concert, inhibit germination and seedling development. Depending on the severity of the stress and the genetic background, germination is either delayed or inhibited [2].

To our knowledge, information on the effect of water and temperature on sunflower seed germination appears to be limited, mainly regarding the detection of water availability in the seed using the TKW method [27–29]. In this regard, the present work highlights distinct technological approaches that may be beneficial in the future for germination research and by farmers in critical circumstances to anticipate the sowing date for sunflower crop establishment and successful seed germination. It contains the following primary objectives: (i) to evaluate the effects of temperature on the germination and development of sunflower seedlings at various temperatures and determine the optimal level; (ii) to determine the minimum, optimal, and maximum water requirements for germination based on the volume of water in one-milliliter increments and the weight of one thousand kernels (TKW), as determined by prior studies [27–29]; (iii) to determine the optimal seed number

and seedling density, as well as the impact of pre-sowing seed technology on seedling development, so as to control fungal growth. Regardless of environmental parameters, sowing date, or plant density, germination tests with varying water and temperature levels and seed numbers can establish all the necessary conditions for effective sunflower germination.

2. Material and Methods

The experiment was caried out on sunflower seeds (*Helianthus annus* L. Larissza) at the Institute of Agronomy (Hungarian University of Agriculture and Life Sciences), 47°35′37″ N, 19°21′55″ E. The experiment was conducted in 2022. In this work, we investigated the effects of abiotic stressors (water and temperature), seedling density, and fungal growth control on seed germination and seedling development in vitro using growth or climate chamber ICO105 (Memmert GmbH + Co. KG, Schwabach, Germany).

2.1. Temperature Test

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 PD 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 re-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. Additionally, germination percentages were determined (Figure 1).

Figure 1. Photos showing seed germination at 25 °C in PD. At this temperature, both germination and seedling development were observed to be at a high level.

2.2. Water Test

The germination response of sunflower seeds to water stresses 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 on the base of the TKW as a percentage (Table 1). 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 (1) was used to compute the amount of water based on Thousand Kernel Weight (TKW) [27,28]:

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

The TKW of the sunflower seeds was 50.46 g. Additional information on calculating water quantity based on TKW is available in previous works [27,28]. The PD were 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).

Table 1. The water levels based on the milliliter intervals of the bases and TKW%.

Amount of Water Based on 1 mL Interval		Amount of Water Based on the TKW %			
[1] TN	[2] WA (mL)	[3] TN	[4] PW %	[5] WA (mL)	[6] RAW (mL)
1	0	14	125	0.631	0.63075
2	1	15	250	1.262	1.2615
3	2	16	375	1.892	1.89225
4	3	17	500	2.523	2.523
5	4	18	625	3.154	3.15375
6	5	19	750	3.785	3.7845
7	6	20	875	4.415	4.41525
8	7	21	1000	5.046	5.046
9	8	22	1125	5.677	5.67675
10	9	23	1250	6.308	6.3075
11	10	24	1375	6.938	6.93825
12	11	25	1500	7.569	7.569
	12	26	1625	8.200	8.19975
		27	1750	8.831	8.8305
		28	1875	9.461	9.46125
		29	2000	10.092	10.092
		30	2125	10.723	10.72275
		31	2250	11.354	11.3535
		32	2375	11.984	11.98425
		33	2500	12.615	12.615

[1] the number of treatments determined by the milliliter technique; [2] the volume of water based on a single milliliter; [3] the number of treatments based on the TKW approach; [4] the suggested percentage for water quantity application in ml regarding the TKW method; [5] the quantity of water equaled the suggested proportion of water volume based on TKW; [6] round the water volume in milliliters on the pipette to the closest discernible digit.

2.3. Seed Number Test

This part of the experiment examined the effect of seed number 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).

2.4. Antifungal Test

The efficiency of the fungicide in inhibiting fungal growth was evaluated using two alternative antifungal application methods. The growth media were treated with five different concentrations of Amistar Xtra in the first method: 0, 20, 200, 2000, and 20,000 ppm. Additionally, two different seed sterilization techniques were examined: the first consisted of soaking the seeds in a 2000 ppm Amistar Xtra solution for three minutes, and the second involved 10% sodium hypochlorite (NaClO) with the same method. The seeds were sterilized, rinsed with distilled water, and then incubated in growth chamber for 10 days at

25 °C. After ten days of incubation, the radicle and shoot lengths were measured, together with the number of seeds that had germinated. This experiment was performed ten times.

2.5. Statistical Analyses

A Completely Randomized Design (CRD) was used for the factorial experiments with replications to examine the effects of temperature levels, water amounts, seed density, and fungal growth control on seed germination and seedling development. The acquired data were analyzed using analysis of variance (ANOVA) and Fisher's test for Least Significant Differences (LSD). Using SPSS V27 (IBM, New York, NY, USA), the Kolmogorov–Smirnov and Shapiro–Wilk test [30,31] were utilized to validate the normality of the data. The effects of water level, seed number, and antifungal treatment on germination percentage, radicle length, shoot length, and total seedling length were analyzed using computer software (GenStat twelfth edition, GenSat procedure library release PL20.1m, and MS Excel 365). A statistical tool was used to fit the data and draw the proper temperature levels using a sigmoid curves model (MS Excel 365 and JMP Pro 13,2,1 of SAS Institute, Cary, NC, USA).

3. Results

3.1. Temperature Effect

The time course of the germination of the sunflowers was conducted at temperatures ranging from 5 to 40 °C, with 5 Celsius intervals (Figure 2). 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. 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, taking approximately 19 days at temperatures 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. Hence, their position on the growth curve is zero (Figure 2).

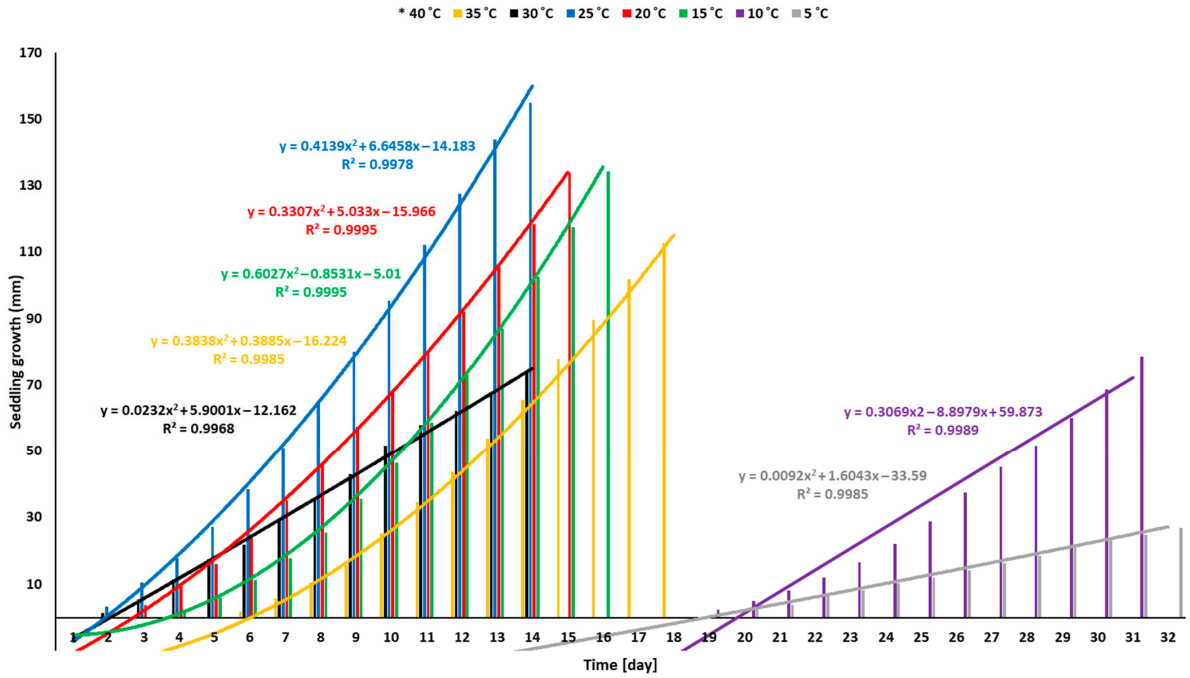


Figure 2. Germination and seedling growth of sunflower seeds vs. time at different levels of temperature. The * represents 40 °C which is not shown on the graph because its value is zero (no germination).

The recording data of the radicle and shoot growth patterns under different temperature levels are presented in Figures 2 and 3. Radicles and shoots can grow at a range of temperatures, although 15 °C, 20 °C, 25 °C, and 35 °C are optimal. The shoot grew optimally at 25 °C (Figures 3 and 4). At the same time, the radicle grew optimally at a temperature between 15 and 25 °C. At 10 °C, radicle growth was more significant and rapid than shoot growth. The radicle appeared to have more distinct temperature requirements than the shoot; its development was more favorable at lower temperatures. Beyond the ideal range, the growth of the two organs accelerated until they reached their maximum height, at which time it leveled off. The shoot and radicle, particularly the radicles, do not require prolonged exposure to elevated temperatures. In addition, their growth was poor at 5 °C and nonexistent at 35 °C (Figures 3 and 4).

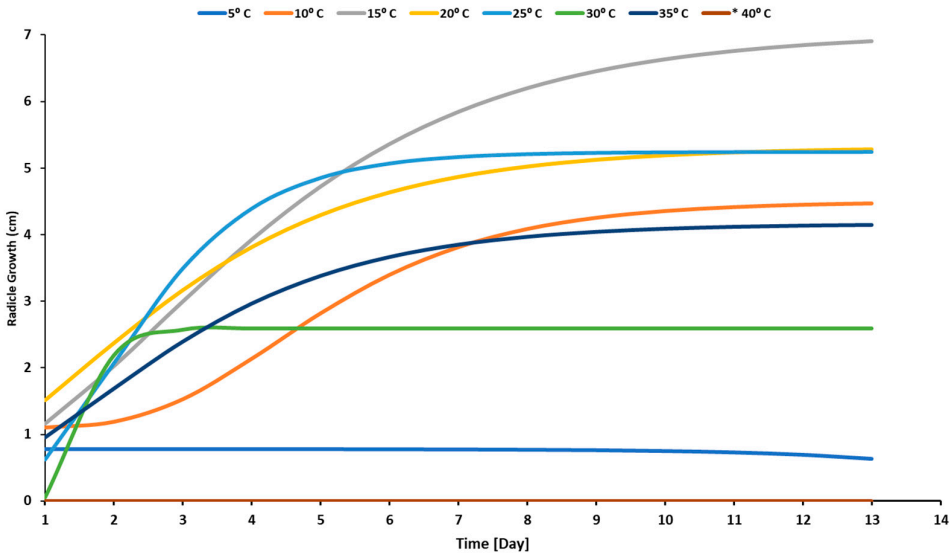


Figure 3. Effect of temperature levels on the growth response of radicle. The * represents 40 °C which is not shown on the graph because its value is zero (no radicle development).

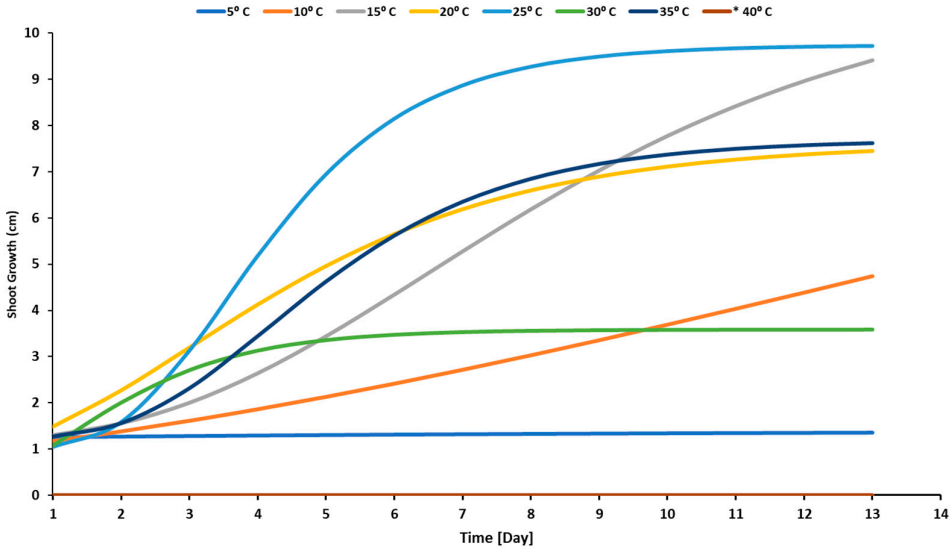


Figure 4. Effect of temperature levels on the growth response of shoot. The * represents 40 °C which is not shown on the graph because its value is zero (no shoot growth).

Figure 4 illustrates the biological effects of different temperatures on the development of the seedling. The results indicated that growth was highest at 25 °C, followed by 15 °C, 20 °C, and 35 °C (Figure 5). Therefore, temperature of 25 degrees Celsius was

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, seedling 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 optimal were deleterious to seedling development (Figure 5).

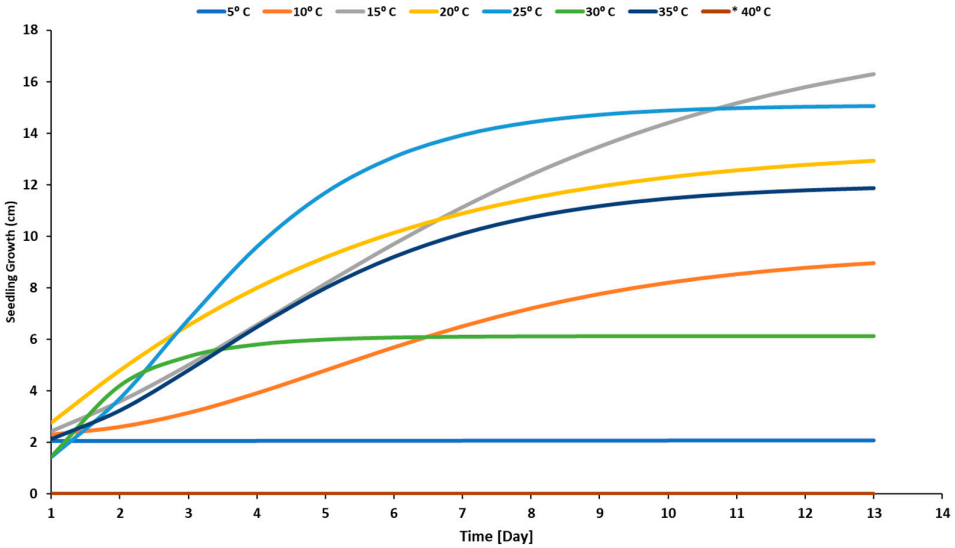
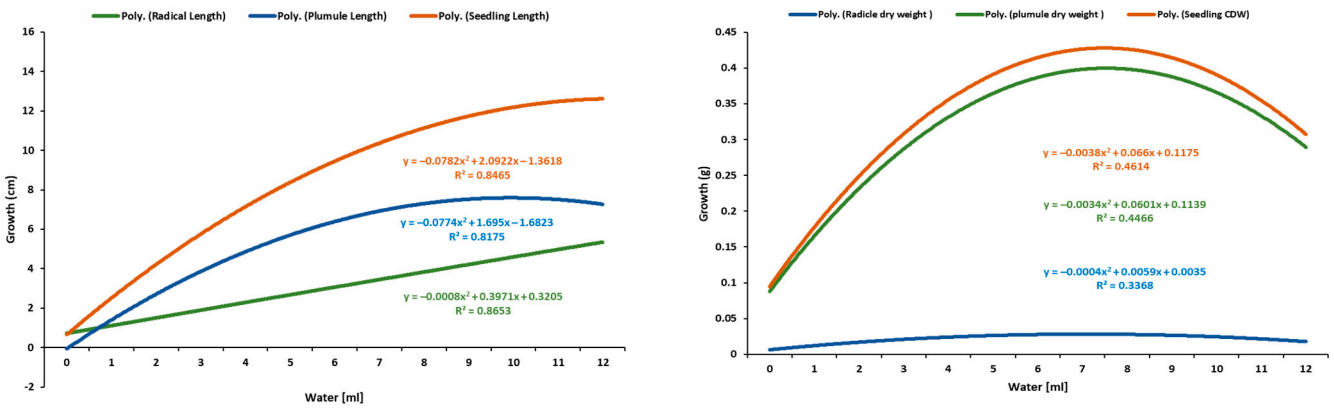


Figure 5. Effect of temperature levels on seedling growth response. The * represents 40 °C which is not shown on the graph because its value is zero (no seedling growth).

3.2. Water Effect

The summary results of the growth indices and dry weight accumulation in response to two water bases, of a single milliliter, and TKW percentage are presented in Tables 2 and 3 and Figures 6 and 7. The statistical analysis based on the LSD values of germinated seed numbers is shown in Tables 2 and 3. There was no significant difference in the number of germinated seeds between different water levels for both methods. As a result, the sunflower seeds germinated successfully at all tested water levels (Tables 2 and 3).



(**a**) (**b**)

Figure 6. The effect of water quantity on seedlings, shoots, and roots. (**a**) Growth in relation to varied amounts of water at 1 mL intervals. (**b**) Dry weight accumulation vs. various quantities of water at 1 mL intervals.

Table 2. Mean values on germination and seedling growth traits for the various amounts of water based on 1 mL intervals.

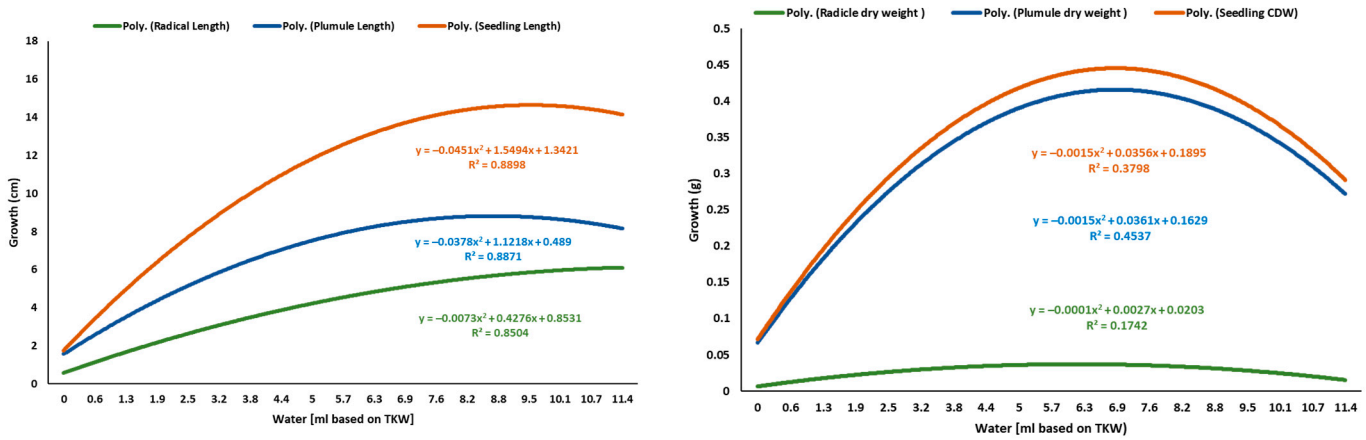
Water mL	Germinated *n*	Radicle L (cm)	Shoot S (cm)	Seedling L (cm)	Shoot DW (g)	Radicle DW (g)	Seedling DW (g)
0	0.0 ± 0.0	0.000 ± 0.00 g	0.000 ± 0.00 g	0.000 ± 0.00 f	0.000 ± 0.00 d	0.000 ± 0.00 f	0.000 ± 0.00 d
1	10.0 ± 0.0	1.860 ± 0.54 f	1.622 ± 0.05 f	3.482 ± 0.55 e	0.351 ± 0.01 abc	0.016 ± 0.00 de	0.367 ± 0.01 abc
2	10.0 ± 0.0	1.844 ± 0.67 f	2.368 ± 0.55 ef	4.212 ± 1.14 e	0.348 ± 0.01 abc	0.024 ± 0.00 bc	0.372 ± 0.02 abc
3	10.0 ± 0.0	1.770 ± 0.67 f	3.096 ± 0.85 e	4.866 ± 1.44 e	0.316 ± 0.07 c	0.026 ± 0.01 bc	0.342 ± 0.07 c
4	10.0 ± 0.0	2.216 ± 0.19 f	5.462 ± 0.44 cd	7.678 ± 0.51 d	0.367 ± 0.02 ab	0.036 ± 0.00 a	0.404 ± 0.03 a
5	10.0 ± 0.0	2.474 ± 0.67 f	5.834 ± 1.22 cd	8.308 ± 1.84 d	0.339 ± 0.03 abc	0.018 ± 0.01 bcde	0.358 ± 0.03 bc
6	10.0 ± 0.0	3.506 ± 0.28 de	6.962 ± 0.77 b	10.468 ± 0.94 bc	0.335 ± 0.03 abc	0.021 ± 0.01 bcd	0.356 ± 0.03 bc
7	10.0 ± 0.0	2.612 ± 0.47 ef	5.506 ± 1.09 cd	8.118 ± 1.45 d	0.341 ± 0.02 abc	0.017 ± 0.01 cde	0.359 ± 0.02 bc
8	10.0 ± 0.0	4.064 ± 0.78 cd	8.996 ± 0.85 a	13.060 ± 1.55 a	0.340 ± 0.03 abc	0.034 ± 0.01 a	0.373 ± 0.03 abc
9	10.0 ± 0.0	5.140 ± 0.83 ab	8.918 ± 0.68 a	14.058 ± 0.93 a	0.328 ± 0.02 bc	0.034 ±0.01 a	0.363 ± 0.02 abc
10	10.0 ± 0.0	3.686 ± 0.65 cd	5.102 ± 0.98 d	8.788 ± 1.46 cd	0.363 ± 0.04 ab	0.014 ± 0.00 e	0.377 ± 0.04 abc
11	10.0 ± 0.0	4.648 ± 1.72 bc	6.380 ± 1.70 bc	11.028 ± 3.40 b	0.372 ± 0.05 a	0.019 ± 0.01 bcde	0.391 ± 0.04 ab
12	10.0 ± 0.0	5.832 ± 1.14 a	8.706 ± 0.80 a	14.538 ± 1.42 a	0.346 ± 0.02 abc	0.026 ± 0.00 b	0.372 ± 0.02 abc
LSD	NS	0.994	1.222	1.906	0.040	0.0075	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.

Table 3. Mean comparison of germination and seedling growth characteristics for varying amounts of water depending on TKW%.

Water mL of the TKW	Germinated *n*	Radicle L (cm)	Shoot S (cm)	Seedling L (cm)	Shoot DW (g)	Radicle DW (g)	Seedling DW (g)
0	0.0 ± 0.00	0.000 ± 0.00 i	0.000 ± 0.00 h	0.000 ± 0.00 j	0.000 ± 0.00 g	0.000 ± 0.00 i	0.000 ± 0.00 f
0.6	10.0 ± 0.00	2.412 ± 0.62 h	4.182 ± 0.34 g	6.594 ± 0.88 i	0.324 ± 0.02 f	0.037 ± 0.01 abcd	0.360 ± 0.03 e
1.3	10.0 ± 0.00	2.804 ± 0.57 gh	3.208 ± 0.17 g	6.012 ± 0.63 i	0.338 ± 0.01 def	0.030 ± 0.00 ef	0.368 ± 0.01 de
1.9	10.0 ± 0.00	3.102 ± 0.50 gh	4.388 ± 0.60 g	7.490 ± 0.19 hi	0.346 ± 0.02 bcde	0.039 ± 0.00 ab	0.384 ± 0.01 abcde
2.5	10.0 ± 0.00	2.782 ± 0.34 gh	5.912 ± 0.70 f	8.694 ± 0.46 gh	0.333 ± 0.02 ef	0.040 ± 0.00 a	0.374 ± 0.02 bcde
3.2	10.0 ± 0.00	2.448 ± 0.32 h	6.008 ± 0.31 f	8.456 ± 0.61 gh	0.351 ± 0.02 abcde	0.024 ± 0.00 fgh	0.374 ± 0.02 bcde
3.8	10.0 ± 0.00	3.338 ± 0.80 fgh	6.136 ± 1.21 f	9.474 ± 1.75 g	0.348 ± 0.04 def	0.038 ± 0.01 abc	0.386 ± 0.04 abcde
4.4	10.0 ± 0.00	4.508 ± 0.93 de	7.448 ± 0.90 cde	11.960 ± 1.81 def	0.338 ± 0.03 abc	0.043 ± 0.00 a	0.381 ± 0.03 abcde
5.0	10.0 ± 0.00	3.468 ± 1.46 fg	6.778 ± 2.85 ef	10.246 ± 4.24 fg	0.367 ± 0.02 a	0.032 ± 0.01 cde	0.399 ± 0.02 ab
5.7	10.0 ± 0.00	4.852 ± 0.57 cde	8.59 ± 0.32 abc	13.438 ± 0.68 cd	0.373 ± 0.02 ab	0.031 ± 0.01 cde	0.405 ± 0.01 a
6.3	10.0 ± 0.00	4.218 ± 0.19 ef	8.734 ± 0.84 ab	12.950 ± 0.93 cde	0.369 ± 0.02 abcde	0.026 ± 0.01 efgh	0.396 ± 0.02 abc
6.9	10.0 ± 0.00	4.178 ± 0.46 ef	7.372 ± 1.14 de	11.550 ± 1.31 ef	0.349 ± 0.02 def	0.023 ± 0.01 gh	0.371 ± 0.01 cde
7.6	10.0 ± 0.00	5.056 ± 0.55 cde	8.568 ± 0.51 abc	13.620 ± 0.76 bcd	0.340 ± 0.02 abcd	0.028 ± 0.01 efg	0.368 ± 0.02 de
8.2	10.0 ± 0.00	6.400 ± 1.32 ab	9.326 ± 0.27 a	15.726 ± 1.46 a	0.359 ± 0.02 ab	0.032 ± 0.00 bcde	0.392 ± 0.02 abcd
8.8	10.0 ± 0.00	5.358 ± 0.14 cd	7.86 ± 0.56 bcd	13.21 ± 0.62 cde	0.369 ±0.02 abcde	0.023 ± 0.00 gh	0.391 ± 0.02 abcd
9.5	10.0 ± 0.00	6.584 ± 0.80 a	9.496 ± 0.84 a	16.08 ± 1.56 a	0.353 ± 0.01 abcde	0.030 ± 0.00 ef	0.383 ± 0.01 abcde
10.1	10.0 ± 0.00	5.468 ± 0.60 bc	7.478 ± 1.25 cde	12.946 ± 1.26 cde	0.359 ± 0.01 abcd	0.021 ± 0.01 h	0.381 ± 0.01 abcde
10.7	10.0 ± 0.00	5.594 ± 1.47 bc	9.090 ± 0.44 a	14.684 ± 1.84 abc	0.342 ± 0.01 cdef	0.031 ± 0.00 de	0.373 ± 0.01 bcde
11.4	10.0 ± 0.00	6.834 ± 0.33 a	8.53± 0.42 bcd	15.360 ± 0.69 ab	0.354 ± 0.03 abcde	0.024 ± 0.00 fgh	0.378 ± 0.02 bcde
LSD	NS	0.947	1.189	1.830	0.025	0.006	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.



(**a**) (**b**)

Figure 7. The influence of water quantities on seedling, plumule, and radicle growth. (**a**) Length vs. water according to TKW%. (**b**) Dry weight buildup versus water according to TKW%.

The growth of radicles, shoots, and seedlings increased significantly with water volume to the optimal level, but decreased significantly as the water quantity increased beyond the optimal level (Figures 6a and 7a; Table 3). The optimal water range for radicle, shoot, and seedling growth was 8.2–11.4, representing 1625–2250 percent of the TKW (Table 3). It was within the ideal range indicated by the one-milliliter (8–12 mL) water-based approach (Table 2). Therefore, we can say that the TKW approach is more precise in optimizing germination water requirements.

Tables 2 and 3 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 effect between the various water levels. The dry weight of radicles and shoots increased significantly as the water quantity increased, stabilized at its optimal level, and then decreased slightly, despite the increased water volume (Figures 6b and 7b; Tables 3 and 4). The optimal range of dry matter in radicles was evaluated to be 1.9–4.4 mL, corresponding to 375–875% of the TKW. Due to its larger structure, the shoot accumulates more dry matter than the root. In addition, the optimal range of dry matter content for the entire seedling corresponded to the optimal range estimated for shoot structure, which was evaluated to be 4.4–8.4 mL, corresponding to 875–1750% of the TKW (Table 3). When the availability of water exceeded 8.8 mL per plant, the dry weight of the seedlings fell (Figures 5b and 6b; Tables 3 and 4).

Table 4. Collecting data on sunflower germination and seedling response to seed density.

Seed Number	Radical cm	Shoot (cm)	Seedling (cm)	Radicle DW (g)	Shoot DW (g)	Seedling DW (g)
6	4.277 ± 1.19 ab	5.673 ± 0.53 ab	9.950 ± 1.60	0.210 ± 0.01 d	0.036 ± 0.01 c	0.246 ± 0.02 d
8	4.846 ± 0.99 a	5.438 ± 0.55 b	10.28 ± 1.34	0.275 ± 0.02 c	0.056 ± 0.01 b	0.331 ± 0.02 c
10	4.420 ± 1.02 ab	5.6 ± 0.27 b	10.04 ± 1.12	0.344 ± 0.01 b	0.071 ± 0.01 a	0.416 ± 0.02 b
12	3.640 ± 0.46 b	6.004 ± 0.37 a	9.644 ±0.61	0.407 ± 0.02 a	0.076 ± 0.01 a	0.483 ± 0.02 a
LSD	0.785	0.335	N.S	0.013	0.007	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. DW: dry weight.

3.3. Seed Number Effect

The effect of seed number on the growth parameters and dry weight accumulation of radicles, shoots, and seedlings is displayed in Table 4. The results indicated a significant difference in the radicle growth, shoot growth, radicle dry weight, shoot dry weight, and

dry seedling weight the 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 4). Therefore, the seed densities of 10–12 appeared to be ideal for developing a sunflower crop in vitro.

3.4. Antifungal Effect

Figure 8 illustrates the recorded data and comparison results for pre-treated germination seeds with antifungal Amistar Xtra and Hypo (10% Sodium hypochlorite (NaClO)). The priming of seeds with Hypo had a significant effect on radicle growth compared to the control, but the antifungal growth medium Amistar Xtra suppressed fungal growth together with radicle, shoot, and seedling growth. Consequently, their development was decreased by 80–86% relative to the control. In addition, the findings given in Figure 9 demonstrated that, even at low doses, all growth parameters dropped correspondingly as antifungal concentrations increased. Figure 10 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.

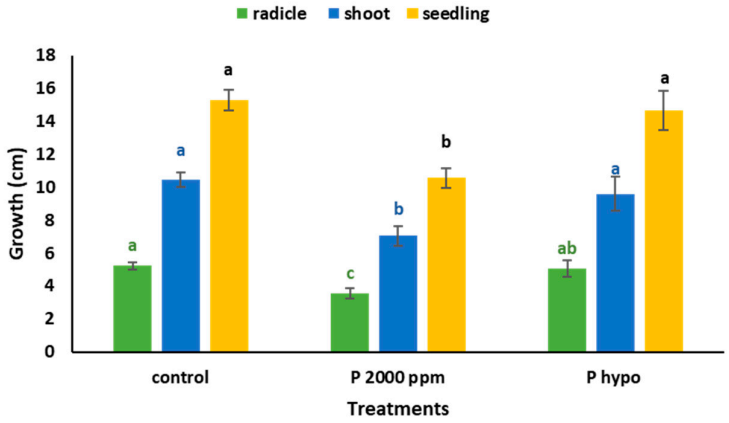


Figure 8. The growth response of the radicle, shoot, and seedling to two distinct fungal seed treatment procedures. Means are presented ±SD (n = 100). At $p < 0.05$, values between treatments marked by different letters differ significantly. They began in order, with the letter (a) being the most significant.

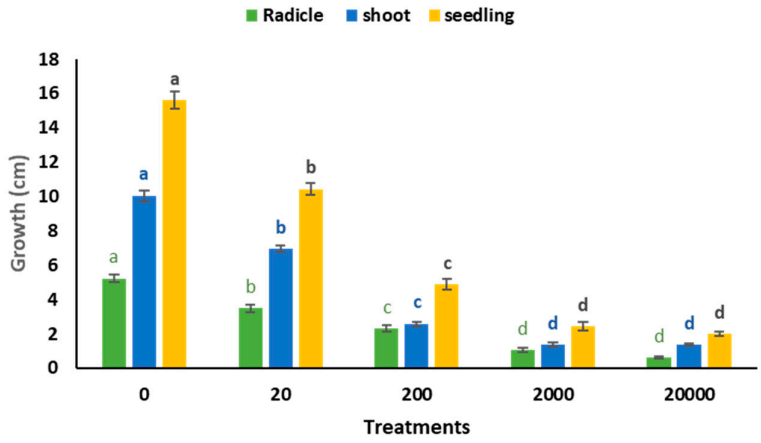


Figure 9. The growth response of radicles, shoots, and seedlings to varying concentrations of the fungicide Amistar Xtra. Means are presented ±SD (n = 100). At $p < 0.05$, values between treatments marked by different letters differ significantly. They began in order, with the letter (a) being the most significant.

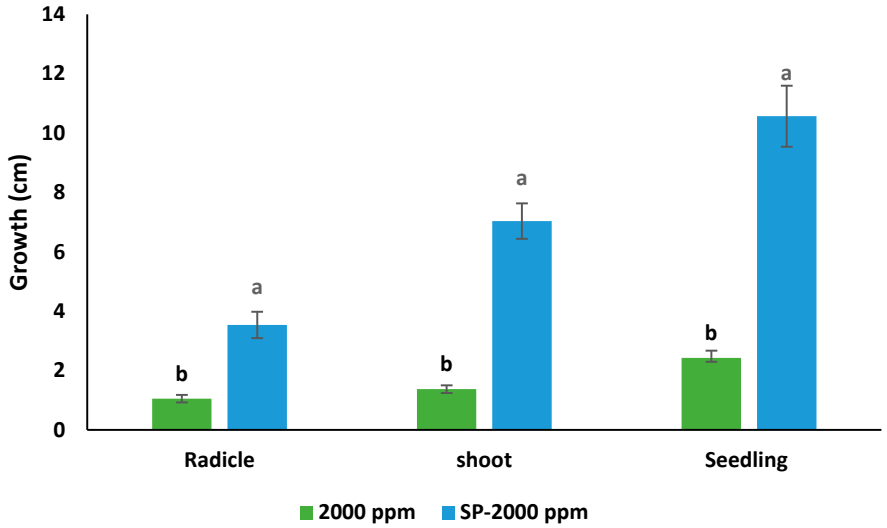


Figure 10. Responses of radicle, plumule, and seedling to two methods of fungicide control (sterilization SP-2000 ppm and growth media 2000 ppm). Means are presented ±SD (n = 100). At $p < 0.05$, values labeled with different letters differ significantly. They began in order, with the letter (a) being the most significant.

4. Discussion

4.1. Effect of Temperature Levels on a Radicle, Plumule, and Seedling Development of Sunflower

The right temperature is required for plants to transition from one phase of their life cycle to the next. In this study, seeds were exposed to eight constant temperature levels ranging from 5 to 40 °C to determine the optimal temperature for sunflower germination. At approximately 25–30 °C, germination (radicle reaching 1 cm) began. Below these temperatures, the beginning of germination gradually slowed. In the present research, sunflower seeds germinated at temperatures between 5 °C and 30 °C, and 25 °C was the ideal temperature for germination, with a range of 15–35 °C (Figure 2).

While sunflower seeds can germinate at 35 °C, the optimal germination temperature was 25 °C (Figure 2). This result is consistent with other works, where the optimum temperature was reported to be close to 25 °C [32,33]. At the optimal temperature, a higher germination rate is achievable [6]. In addition, it enables a more efficient combination of germination rate and percentage [34]. The significant reduction in germination potential and germination energy above or below 25 °C indicates the existence of a high- and low-cut threshold between the temperature range of studies.

Low temperatures of 5 °C or below delayed the germination process and meant this took a longer time (Figure 2). Higher temperatures accelerated the germination process [35,36]. Low and critical high temperatures slowed the metabolic rate to the point where essential pathways for germination initiation ceased to function. This can lead to a loss of seed viability and poor seedling development. Furthermore, elevated temperatures cause plants to have a faster metabolism, which depletes the seed energy required for growth [8]. Temperature promotes metabolic and enzyme activity in addition to the seed's ability to absorb water, hence enhancing germination [5]. At extremely high temperatures, the available energy for growth is lost rapidly, preventing germination [36]. In addition, when the temperature rises, the growth of the radicle (Figure 3) and the shoot (Figure 4) increases linearly until the optimal level is reached, after which it decreases linearly. The optimal temperature range for radicles was determined to be 15–35 °C, with temperatures of 15 and 25 °C overlapping in the early stages (Figure 3). Each development stage's temperature requirements contribute to the radicle's temperature sensitivity. Previous research has demonstrated that each growth phase of the radicle requires a specific cumulative temperature [27,28].

Not only does germination need a certain temperature, but so do root development and shoot growth at each stage. For example, the plumule grew similarly between 15 and 35 ° C (Figure 4). However, it required a higher temperature than the radicle, which had a higher length value at 25 ° C (Figure 4). A shoot in the late stages showed an overlapping growth pattern between 15 and 20 °C. The same finding was observed in the seedling development during the very late stages, between the temperatures of 15 and 25 °C (Figure 5). As each part of the stem and root responds differently to temperature, evaluating the complete seedling development is a more reliable method for predicting the effects of temperature on plants. The optimal temperature range for sunflower germination and seedling development was determined to be 25 °C, with a range of 15 to 35 °C. The temperature of 40 °C had a negative effect on them (Figures 2 and 5); most enzymes became inactive, and seeds died, indicating that the high temperature accelerated the seeds' aging [8].

4.2. Effect of Water Levels on the Radicle, Plumule, and Seedling Development of Sunflower

This study examines the effect of one of the most influential environmental factors on germination, water, which influences sunflower establishment from sowing to seed filling. Water absorption is a prerequisite for germination. The amount of water substantially affected the seed germination potential (Tables 2 and 3).

According to both methods, with the one-milliliter intervals technique and TKW water levels, due to their deep and long root, sunflower seeds could germinate at low and higher water availability, beginning at 0.6 mL, or 125% (Table 2). This quantity may be equal or close to the required moisture content for germination. In the literature, this proportion was estimated to be 30% for corn, 40% for wheat, and 50% for soybeans [37]. Species diversity may be responsible for these differences. Once the critical seed moisture content is reached, sufficient moisture is present to initiate germination. Previous research has documented the minimum water potential required to induce seed imbibition [38,39] during the plateau phase of seed imbibition, a crucial phase in regulating seed germination. It was observed that the duration of this phase is highly dependent on water potential and species. It can be extended to water potentials around −0.03 MPa in some species [38] and −1.0 MPa in others [39].

Generally, an optimal quantity of germination water has a substantial impact on plant growth. Water availability is advantageous to the shoot, radicle, and seedling. Although the radicle grows more slowly than the shoot, their optimal water conditions for growth and development are similar. As a result, the optimal water range for total seedling length is 8.2 to 11.4 mL, corresponding to 1625 to 2250% of the TKW. Radicle growth is the most critical factor in early seedling survival because it enables the seedling to exploit soil water through rapid root extension [40]. Under varying water conditions, dry weight accumulation in the radicle, shoot, and seedling revealed a significant difference (Tables 2 and 3). The optimal water range for radicle development was determined to be 8.2–11.4 mL, representing 1625–2250% of the TKW (Table 3). This value was higher than their dry matter accumulation: approximately 1.9 and 4,4 mL, representing 375–875% of the TKW (Table 3). This finding demonstrated that the sunflower's prominent radicle structure allows it to exploit available water. However, water application that exceeded the optimal range had a detrimental effect on the dry matter accumulation of plants.

Given that rapid radicle development under optimal hydric conditions contributed significantly to shoot dry matter accumulation, the plant will respond to increased water availability by increasing its flow and assimilating plumule to increase its dry matter accumulation. As a result, the accumulated dry matter for the plumule was determined to be between 4.4 and 8.2 milliliters, representing 875 to 1625% of the TKW. Furthermore, the radicle structure required less water to produce dry matter: 1.9–4.4 mL (Table 3). Therefore, the optimal range for the entire seedling 4.4–8.4 mL, as determined by the TKW method, which falls within the optimal range determined by the one-milliliter method

(4–12 mL), confirming the TKW method's accuracy in determining the minimum optimal water requirement for germination, as supported by previous research [27–29].

4.3. Effect of Seed Number Density on Seed Germination and Growth Traits

In this experiment, we compared the effects of 6, 8, 10, and 12 sets of seeds per PD on germination and growth parameters to determine the optimal number of seeds that results in the highest germination efficiency. The results revealed a significant difference in the growth and dry weight of the radicle and shoot among the seed numbers set (Table 4). The seedling growth and dry weight increased as the number of seeds per Petri dish increased. High seedling length and dry seedling matter were observed using a set of 10–12 seeds per PD. Although 6–8 seed densities enhanced germination, seedling development was slower [41]. 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 [42,43]. In addition, dense seedlings could be more susceptible to lodging, which increases disease incidence [44] and, as a result, the seedling emergence percentage [45]. The optimal seed density for growing sunflowers in vitro under controlled conditions is 10–12 seeds per PD (Table 4). Therefore, optimizing seed density per PD and other environmental factors is crucial during germination tests.

4.4. Effect of Antifungal Experiment on a Radicle, Plumule, and Seedling Development

The antifungal Amistar Xtra had a negative effect on radicle, shoot, and seedling development, given that its primary function is to inhibit fungal growth (Figure 8); these findings corroborate that of previous works [27–29]. It has the active component azoxystrobin, which blocks electron transport in the mitochondrial respiratory chain of fungi, hence decreasing aerobic energy production and slowing the growth of fungi [46]. Additionally, as the antifungal concentration in the growth media increases, the inhibitory effect becomes more significant (Figure 9). The absence of fungi in our experiment could have been the cause, rather than the fungicide's phytotoxicity, as many authors suggest in their works [47–49]. In addition, they observed a recovery in seedling growth after fungal exposure and inoculation. Priming seeds with Amistar Xtra or Hypo, as opposed to amending seeds in antifungal growth conditions, was observed to reduce in vitro fungus development (Figures 8 and 10).

5. Conclusions

It is crucial to consider the length of the vegetative season and the plant's ability to germinate when planning the cultivation of sunflowers. This would allow farmers to schedule the planting date, assuring the crop's successful establishment and growth. In this study, the optimal temperature range for sunflower germination and seedling development was determined to be 25 °C, with a range of 15 and 35 °C. This finding revealed that optimal seed germination could occur as spring temperatures rise before the hot temperatures of summer. Below or above optimum ranges, the germination potential was diminished. The water has a significant effect on sunflower germination and growth. The hybrid utilized in this experiment had the potential to germinate in a wide range of water concentrations, beginning at 0.6 mL, or 125% of the TKW. Optimizing the water availability for seeds based on milliliters of the TKW appeared to be the most successful strategy since it takes into consideration the seed's weight and size when estimating the appropriate amount of water for optimal seed development. The optimal amount of water for seed germination, seedling development, and dry matter buildup ranged from 4.42 to 8.2 mL, or 875 to 1625% of the TKW. In addition, regulating seedling density is essential to prevent water factor limitation and competition among seedlings, as well as to limit disease issues. Therefore, 1012 seeds per Petri dish was found to be the optimal seed density for cultivating sunflowers in vitro. In terms of antifungal control, priming seeds with an antifungicide to suppress fungal growth is recommended. Future studies, breeding initiatives, and the prediction of sowing dates may benefit from these findings, particularly when seed supplies are limited. Seed priming is a more effective antifungal application technique than other techniques.

Author Contributions: Conceptualization, Á.T. and Z.K.; methodology, writing—original draft preparation A.H.S.; software and investigation, H.K.; writing—review and editing, H.K.; funding acquisition, C.G. and G.P.K. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the framework of the Thematic Excellence Program 2021, National Defense and National Security Subprogramme (TKP2021-NVA-22) in accordance with the Hungarian University of Agriculture and Life Sciences.

Data Availability Statement: All data, tables, and figures in this manuscript are original.

Acknowledgments: The authors express their sincere thanks to the Stipendium Hungaricum Foundation and all the colleagues of the Institute of Agronomy of the Hungarian University of Agriculture and Life Sciences for their support in this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Aubertot, J.N.; Deguine, J.P.; Lamichhane, J.R.; Robin, M.H.; Sarthou, J.P.; Steinberg, C. Vers Une Protection Agroécologique Des Cultures En Phase d'implantation. In *Réussir l'Implantation Des Cultures. Enjeux Agroécologiques, Itinéraires Techniques*; Quae: Paris, France, 2022; pp. 107–134.
2. Ali, A.S.; Elozeiri, A.A. Metabolic Processes During Seed Germination. In *Advances in Seed Biology*; InTech: Vienna, Austria, 2017.
3. Gummerson, R.J. The Effect of Constant Temperatures and Osmotic Potentials on the Germination of Sugar Beet. *J. Exp. Bot.* **1986**, *37*, 729–741. [CrossRef]
4. Maraghni, M.; Gorai, M.; Neffati, M. Seed Germination at Different Temperatures and Water Stress Levels, and Seedling Emergence from Different Depths of *Ziziphus lotus*. *S. Afr. J. Bot.* **2010**, *76*, 453–459. [CrossRef]
5. de Oliveira, A.K.M.; Ribeiro, J.W.F.; Pereira, K.C.L.; Silva, C.A.A. Efeito Da Temperatura Sobre a Germinação de Sementes de *Diptychandra aurantiaca* (Fabaceae). *Acta Sci. Agron.* **2013**, *35*, 203–208. [CrossRef]
6. Alvarado, V.; Bradford, K.J. A Hydrothermal Time Model Explains the Cardinal Temperatures for Seed Germination. *Plant Cell Environ.* **2002**, *25*, 1061–1069. [CrossRef]
7. Motsa, M.M.; Slabbert, M.M.; van Averbeke, W.; Morey, L. Effect of Light and Temperature on Seed Germination of Selected African Leafy Vegetables. *S. Afr. J. Bot.* **2015**, *99*, 29–35. [CrossRef]
8. Hawker, J.; Jenner, C. High Temperature Affects the Activity of Enzymes in the Committed Pathway of Starch Synthesis in Developing Wheat Endosperm. *Funct. Plant Biol.* **1993**, *20*, 197. [CrossRef]
9. Nykiforuk, C.L.; Johnson-Flanagan, A.M. Germination and Early Seedling Development under Low Temperature in Canola. *Crop Sci.* **1994**, *34*, 1047–1054. [CrossRef]
10. Hoppe, A.; Theimer, R.R. Degradation of Oil Bodies Isolated from Cotyledons during Germination of Rapeseed Seedlings. *J. Plant Physiol.* **1997**, *151*, 471–478. [CrossRef]
11. Muscolo, A.; Sidari, M.; Anastasi, U.; Santonoceto, C.; Maggio, A. Effect of PEG-Induced Drought Stress on Seed Germination of Four Lentil Genotypes. *J. Plant Interact.* **2014**, *9*, 354–363. [CrossRef]
12. Khaeim, H.M.; Jeber, B.A.; Ali, M.A. Winter Wheat Genotypes Response to Different Water Quality. *Int. J. Agric. Stat. Sci.* **2019**, *15*, 669–676.
13. el Midaoui, M.; Talouizte, A.; Benbella, M.; Serieys, H.; Griveau, Y.; Bervillé, A. Effect of osmotic pressure on germination of sunflower seeds (*Helianthus annuus* L.). *Helia* **2001**, *24*, 129–134. [CrossRef]
14. Wen, B. Effects of High Temperature and Water Stress on Seed Germination of the Invasive Species Mexican Sunflower. *PLoS ONE* **2015**, *10*, e0141567. [CrossRef]
15. Soleimanzadeh, H. Response of Sunflower (*Helianthus annuus* L.) to Selenium Application under Water Stress. *World Appl. Sci. J.* **2012**, *17*, 1115–1119.
16. de Morais, M.B.; Barbosa-Neto, A.G.; Willadino, L.; Ulisses, C.; Calsa Junior, T. Salt Stress Induces Increase in Starch Accumulation in Duckweed (*Lemna aequinoctialis*, Lemnaceae): Biochemical and Physiological Aspects. *J. Plant Growth Regul.* **2019**, *38*, 683–700. [CrossRef]
17. Kovács, Z.; Bedő, J.; Pápai, B.; Tóth-Lencsés, A.K.; Csilléry, G.; Szőke, A.; Bányai-Stefanovits, É.; Kiss, E.; Veres, A. Ripening-Induced Changes in the Nutraceutical Compounds of Differently Coloured Pepper (*Capsicum annuum* L.) Breeding Lines. *Antioxidants* **2022**, *11*, 637. [CrossRef] [PubMed]
18. Kunos, V.; Cséplő, M.; Seress, D.; Eser, A.; Kende, Z.; Uhrin, A.; Bányai, J.; Bakonyi, J.; Pál, M.; Mészáros, K. The Stimulation of Superoxide Dismutase Enzyme Activity and Its Relation with the *Pyrenophora teres f. teres* Infection in Different Barley Genotypes. *Sustainability* **2022**, *14*, 2597. [CrossRef]
19. Hoekstra, F.A.; Golovina, E.A.; Buitink, J. Mechanisms of Plant Dessication Tolerance. *Trends Plant Sci.* **2001**, *6*, 431–438. [CrossRef]

20. Food and Agriculture Organization of the United Nations. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 20 February 2022).
21. Adeleke, B.S.; Babalola, O.O. Oilseed Crop Sunflower (*Helianthus annuus*) as a Source of Food: Nutritional and Health Benefits. *Food Sci. Nutr.* **2020**, *8*, 4666–4684. [CrossRef]
22. Forleo, M.B.; Palmieri, N.; Suardi, A.; Coaloa, D.; Pari, L. The Eco-Efficiency of Rapeseed and Sunflower Cultivation in Italy. Joining Environmental and Economic Assessment. *J. Clean. Prod.* **2018**, *172*, 3138–3153. [CrossRef]
23. Ullah Khan, S.; Khan, A.; Raza Gurmani, A.; Jalal-Ud-Din, A. Oil yield, fatty acid profile, achene yield and yield attributes of sunflower *(Helianthus annuus* L.) as influenced by autumn planting conditions in Islamabad. *Pak. J. Bot.* **2013**, *45* (Suppl. S1), 107–110.
24. Katsarou, A.I.; Kaliora, A.C.; Papalois, A.; Chiou, A.; Kalogeropoulos, N.; Agrogiannis, G.; Andrikopoulos, N.K. Serum Lipid Profile and Inflammatory Markers in the Aorta of Cholesterol-Fed Rats Supplemented with Extra Virgin Olive Oil, Sunflower Oils and Oil-Products. *Int. J. Food Sci. Nutr.* **2015**, *66*, 766–773. [CrossRef] [PubMed]
25. Oshundiya, F.O.; Olowe, V.I.O.; Sowemimo, F.A.; Odedina, J.N. Seed Yield and Quality of Sunflower (*Helianthus annuus* L.) as Influenced by Staggered Sowing and Organic Fertilizer Application in the Humid Tropics. *Helia* **2014**, *37*, 237–255. [CrossRef]
26. Hussain, M.; Farooq, S.; Hasan, W.; Ul-Allah, S.; Tanveer, M.; Farooq, M.; Nawaz, A. Drought Stress in Sunflower: Physiological Effects and Its Management through Breeding and Agronomic Alternatives. *Agric. Water Manag.* **2018**, *201*, 152–166. [CrossRef]
27. Khaeim, H.; Kende, Z.; Balla, I.; Gyuricza, C.; Eser, A.; Tarnawa, Á. The Effect of Temperature and Water Stresses on Seed Germination and Seedling Growth of Wheat (*Triticum aestivum* L.). *Sustainability* **2022**, *14*, 3887. [CrossRef]
28. Khaeim, H.; Kende, Z.; Jolánkai, M.; Kovács, G.P.; Gyuricza, C.; Tarnawa, Á. Impact of Temperature and Water on Seed Germination and Seedling Growth of Maize (*Zea mays* L.). *Agronomy* **2022**, *12*, 397. [CrossRef]
29. Haj Sghaier, A.; Tarnawa, Á.; Khaeim, H.; Kovács, G.P.; Gyuricza, C.; Kende, Z. The Effects of Temperature and Water on the Seed Germination and Seedling Development of Rapeseed (*Brassica napus* L.). *Plants* **2022**, *11*, 2819. [CrossRef]
30. Drebee, H.A.; Razak, N.A.A. Measuring the Efficiency of Colleges at the University of Al-Qadisiyah-Iraq: A Data Envelopment Analysis Approach. *J. Ekon. Malays.* **2018**, *52*, 163–179. [CrossRef]
31. Drebee, H.A.; Razak, N.A.A.; Shaybth, R.T. The Impact of Governance Indicators on Corruption in Arab Countries. *Contemp. Econ.* **2020**, *14*, 354–365. [CrossRef]
32. Gay, C.; Corbineau, F.; Come, C. Effects of Temperature and Oxygen on Seed Germination and Seedling Growth in Sunflower (*Helianthus annuus* L.). *Environ. Exp. Bot.* **1991**, *31*, 193–200. [CrossRef]
33. Khalifa, F.M.; Schneiter, A.A.; Eltayeb, E.I. Temperature-germination responses of sunflower *(Helianthus annuus* L.) genotypes. *Helia* **2000**, *23*, 97–104. [CrossRef]
34. Filho, M. *Fisiologia de Sementes de Plantas Cultivadas*; ABRATES: Londrina, Brazil, 2015.
35. Windauer, L.B.; Martinez, J.; Rapoport, D.; Wassner, D.; Benech-Arnold, R. Germination Responses to Temperature and Water Potential in Jatropha Curcas Seeds: A Hydrotime Model Explains the Difference between Dormancy Expression and Dormancy Induction at Different Incubation Temperatures. *Ann. Bot.* **2012**, *109*, 265–273. [CrossRef] [PubMed]
36. Cassaro-Silva, M. Efeito Da Temperatura Na Germinação de Sementes de Manduirana (*Senna macranthera* (Collad.) Irwin et Barn.—Caesalpiniaceae). *Rev. Bras. De Sementes* **2001**, *23*, 92–99. [CrossRef]
37. Vierling, E.; Kimpel, J.A. Plant Responses to Environmental Stress. *Biotechnology* **1992**, *3*, 164–170.
38. Ramírez-Tobías, H.M.; Peña-Valdivia, C.B.; Trejo, C.; Aguirre, R.J.R.; Vaquera, H.H. Seed Germination of Agave Species as Influenced by Substrate Water Potential. *Biol. Res.* **2014**, *47*, 1–9. [CrossRef]
39. Campos, H.; Trejo, C.; Peña-Valdivia, C.B.; García-Nava, R.; Víctor Conde-Martínez, F.; Cruz-Ortega, R. Water Availability Effects on Germination, Membrane Stability and Initial Root Growth of *Agave lechuguilla* and *A. salmiana*. *Flora Morphol. Distrib. Funct. Ecol. Plants* **2020**, *268*, 151606. [CrossRef]
40. Schütz, W.; Lamont, B.B.; Milberg, P. Germination Requirements and Seedling Responses to Water Availability and Soil Type in Four Eucalypt Species. *Acta Oecologica* **2002**, *23*, 23–30. [CrossRef]
41. Linhart, Y.B. Density-Dependent Seed Germination Strategies in Colonizing Versus Non-Colonizing Plant. *J. Ecol.* **1976**, *64*, 375–380. [CrossRef]
42. Callaway, R.M.; Walker, L.R. Competition and Facilitation: A Synthetic Approach to Interactions in Plant Communities. *Ecology* **1997**, *78*, 1958–1965. [CrossRef]
43. White, T.C.R. Opposing Paradigms: Regulation or Limitation of Populations? *Oikos* **2001**, *93*, 148–152. [CrossRef]
44. Glen, D.M.; Milsom, N.F.; Wiltshire, C.W. Effects of Seed-Bed Conditions on Slug Numbers and Damage to Winter Wheat in a Clay Soil. *Ann. Appl. Biol.* **1989**, *115*, 177–190. [CrossRef]
45. Lortie, C.J.; Turkington, R. The Effect of Initial Seed Density on the Structure of a Desert Annual Plant Community. *J. Ecol.* **2002**, *90*, 435–445. [CrossRef]
46. Bertelsen, J.R.; de Neergaard, E.; Smedegaard-Petersen, V. Fungicidal Effects of Azoxystrobin and Epoxiconazole on Phyllosphere Fungi, Senescence and Yield of Winter Wheat. *Plant Pathol.* **2001**, *50*, 190–205. [CrossRef]
47. Vasanthakumari, M.M.; Shridhar, J.; Madhura, R.J.; Nandhitha, M.; Kasthuri, C.; Janardhana, B.; Nataraja, K.N.; Ravikanth, G.; Uma Shaanker, R. Role of Endophytes in Early Seedling Growth of Plants: A Test Using Systemic Fungicide Seed Treatment. *Plant Physiol. Rep.* **2019**, *24*, 86–95. [CrossRef]

48. Verma, S.K.; Kingsley, K.L.; Bergen, M.S.; Kowalski, K.P.; White, J.F. Fungal Disease Prevention in Seedlings of Rice (*Oryza sativa*) and Other Grasses by Growth-Promoting Seed-Associated Endophytic Bacteria from Invasive Phragmites Australis. *Microorganisms* **2018**, *6*, 21. [CrossRef] [PubMed]
49. Verma, S.K.; Kingsley, K.; Irizarry, I.; Bergen, M.; Kharwar, R.N.; White, J.F. Seed-Vectored Endophytic Bacteria Modulate Development of Rice Seedlings. *J. Appl. Microbiol.* **2017**, *122*, 1680–1691. [CrossRef] [PubMed]