



## Article

# Germination of *Triticum aestivum* L.: Effects of Soil–Seed Interaction on the Growth of Seedlings

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**Abstract:** Seed size, sowing depth, and seed disinfection can affect seed germination and seedling establishment, which, in turn, can directly affect crop growth and yield. The current study was comprised of two experiments, the first of which was conducted in the laboratory, and a second which was performed under glasshouse conditions. The objective of these experiments was to investigate the effects of seed size, sowing depth, and seed disinfection on seed germination and initial seedling growth of selected wheat (*Triticum aestivum* L.) cultivars. The treatments in laboratory experiment were arranged in a completely randomized design, which included: (I) four wheat cultivars (Pishgam, Haydari, Soissons, and Mihan), (II) two seed size classes ( $x < 2.25$  mm, and  $x > 2.25$  mm), and two disinfection treatments (no-disinfection and disinfection), (III) with five replicates. In addition to the aforementioned treatments, the effect of planting depth (4, 6, and 8 cm) was also investigated in the subsequent glasshouse experiment. The best results were obtained at a sowing depth of 4 cm, in the non-disinfected treatment, using large seeds. In contrast, the lowest percentage and speed of seed germination and vigor index were observed in seeds sown at 8 cm depth, in the disinfected seed treatment, using small seeds. Large seeds contain larger nutrient stores which may improve seed germination indices, which would therefore result in improved percentage and speed of seed germination, followed by faster coleoptile and seedling growth, higher seedling dry weight and seed vigor. These data also illustrated that seed disinfection in the Pishgam and Haydari cultivars had inhibitory effects upon coleoptile growth and seedling length, which could be related to the fungicide's chemical composition. Unlike other cultivars, disinfection did not show a significant effect on the Soissons cultivar. Based on our data, in order to improve both the speed of wheat seed germination and subsequent plant growth and development; it is necessary to select high-quality, large seeds, planted at a specific planting depth, which have been treated with an effective disinfectant; all of which will be specific for the wheat cultivar in question. Overall, the current study has provided useful information on the effect size seed, sowing depth, and disinfection have upon germination characteristics and seedling growth of wheat cultivars, which can form the basis for future field scale trails.

**Keywords:** soil; wheat; seed size; germination; disinfection

## 1. Introduction

As the most widely cultivated cereal crop, wheat (*Triticum aestivum* L.) is a staple foodstuff worldwide. Despite major increases in yield in recent decades, some problems such as poor seed germination have reduced the yield potential of this crop in some parts of the world [1]. A first step towards alleviating this problem is to enhance our understanding of the factors affecting seed germination and seedling establishment, in conjunction with an assessment of the effect(s) of seed size and seed sowing depth [2–4]. High-quality seed can increase yields by 15–20% [4]. Seeds differ by size, weight, and density due to the production environment and cultivation practices. Seed size is one of the key parameters of seed quality that affects subsequent crop performance [5–7]. Size is a widely accepted measure of seed quality, and large seeds have high survival growth and establishment [8]. A wide array of factors related to seedling growth, including seed germination, emergence, and agronomical aspects, are affected by seed size [9–12]. Many studies have reported a direct correlation between seedling vigor, improved stand establishment, and higher cereal productivity with plants originating from large seeds compared to those grown from smaller seeds [9]. Larger seeds with a higher germination rate produce seedlings with a heightened competitive ability against weeds and pests [13–16]. Indeed, a larger food reserve (endosperm) in the larger seeds results in increased vigor [1,17]. The size of wheat seeds has been shown to influence crop emergence and establishment and also affect a variety of yield components and, ultimately, grain yield [18–20]. In general, plants grown from larger seeds have been shown to outperform plants germinated from small seeds [21–23]. However, these results not only vary widely between crop species, but also between cultivars of an individual species.

The depth at which seed is sown is another critical factor determining subsequent seed germination and seedling establishment. However, opinions on this topic are varied and it is a complex area of research [24–28].

With regards to plant nutrition, in mainstream crop production, nutrient management has focused on enriching the soil surface by fertilization with nutrients. However, the subsoil contains high amounts of nutrients and water that are potentially available for plant use [24–26,29]. In most agroecosystems, greater exploitation of subsoil resources may lead to reduced nutrient resources and more significant sequestration of atmospheric carbon [25–28]. Thus, increasing the rate of root elongation, the maximal rooting depth, and root length density in the subsoil can all provide promising strategies with which to improve the crop productivity [30]. However, several reports in the literature do not consider deep seed sowing beneficial for germination and seedling establishment [31,32]. The increase in coleoptile length before emergence is the most critical indicator of seedling establishment. A recent study has shown that deep seed sowing affects coleoptile growth, and thus the seedlings fail [33]. Kimmelshue and colleagues stated that one of the major problems facing agriculture programs in the Sahel is sowing depth, which imposes high soil pressure during seed germination and shoot up-thrust [34]. Incorrect planting depth of seed is widely considered as one of the most common errors that occur in global agriculture [34]. Alngiemshy and collaborators stated that each plant species has a specific optimal sowing depth requirement based upon the type of seed and the prevailing environmental conditions [32]. Sowing depth is thus an important factor in achieving strong stand establishment and higher crop yields [32]. Plants sown at shallow depths receive inadequate soil moisture in the topsoil layer, resulting in poor germination [35]. Conversely, sowing seed too deep can also significantly reduce germination and growth [31].

From the perspective of plant pathology, healthy seeds are essential to increase production efficiency. Seed health is a well-accepted component in modern agricultural research to achieve the appropriate plant population and yield. Healthy seeds are regarded as those either free of contamination, or which have acceptable levels of contamination, that do not prevent their germination and which can produce strong and healthy seedlings [36].

Numerous disinfection treatments are used on seed surfaces to eliminate pathogenic infection, increase production efficiency, and extend the duration of seed storage [37]. These

treatments include agricultural biological agents [38], hot water immersion, ionizing radiation, radioisotopes, and exposure to laser light [37]. Among the disinfection method used, chemical treatment is a common practice used for surface sterilization/disinfection [37]. However, some studies have reported that using such chemicals as seed disinfectants has delayed germination and seedling establishment.

Here, we report the results of a study, based upon the assumption that planting large seeds of widely used productive cultivars, with the application of appropriate agrochemicals, sown at an optimal depth, all have a major influence on successful seed germination, seedling establishment, and higher yields. In order to test this hypothesis, the current study was conducted on selected cultivars of wheat (*Triticum aestivum* L.) with the following aims: (1) to determine the effect of seed size on seed germination and initial seedling growth, (2) to assess the effect of sowing depth on seed germination and growth-related criteria, and (3) to determine the effect of seed disinfection on the percentage and speed of seed germination and the vigor index.

## 2. Materials and Methods

For the current study, two sets of experiments were conducted, one under laboratory conditions and the second under glasshouse conditions. Both sets of experiments were performed at the Agricultural and Natural Resources Research and Education Center of Hamadan (ANRRECH), Iran (34.8673° N, 48.5379° E, 1850 m).

### 2.1. Wheat Cultivars

Four wheat cultivars were selected for use in our study, namely: Pishgam, Haydari, Mihaan, and Soissons, seed for which was obtained from the Agricultural and Natural Resources Research and Education Center of Hamadan (ANRRECH), Iran. The average seed size in the wheat cultivars used was approximately 2.25 mm. The seeds were then graded into two groups by sieving, namely, large ( $x > 2.25$  mm), and small ( $x < 2.25$  mm).

### 2.2. Laboratory Experiment

All seeds were surface-sterilized using hypochlorite solution (3%v/v) for 30 s, followed by washing three times with distilled water.

For each treatment, 100 seeds were placed in a plastic container on top of two layers of filter paper. Ten milliliters of sterile distilled water (SDW) were then added to each container, in order to maintain the moisture level during the experiment. The containers were incubated at 25 °C, in a germinator for 10 days, until coleoptile elongation had ceased (see Figure 1). This was determined by monitoring until the first leaf had protruded from the tip of the coleoptiles [39,40]. The final lengths of the coleoptiles were then measured. This technique is widely used for the determination of seed germination and coleoptile length in seed certification tests. A  $4 \times 2 \times 2$  completely randomized factorial design, with five replications, was utilized for this experiment. The first factor consisted of the 4 wheat cultivars. The second factor included the two seed sizes, i.e., large ( $>2.25$  mm) and small ( $<2.25$  mm) seed size. The third factor involved two levels of seed treatment with fungicide and its related control (without fungicide).



**Figure 1.** First experiment. Root emergence (A), root elongation (B), and excised root and stem preparations for subsequent measurements (C).

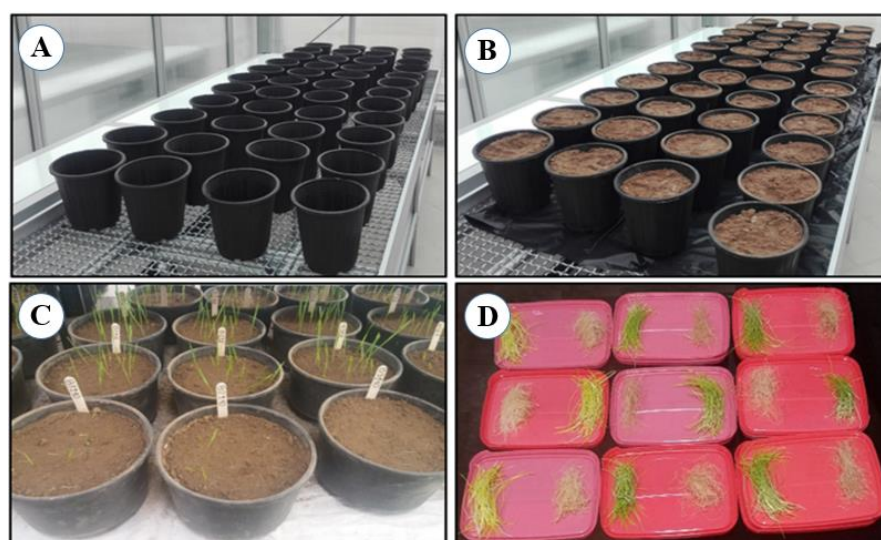
### 2.3. Glasshouse Experiment

In the glasshouse experiment, the treatments were arranged in a completely randomized design, which included (I) four wheat cultivars, (II) two seed size classes, (III) two disinfection treatments, and (IV) three planting depths (4, 6, and 8 cm) with five replications. Sixteen seeds of each seed size of each cultivar were sown at 4, 6, and 8 cm depth in plastic pots (30 cm diameter). Each pot contained 4 kg of soil that was prepared at the ANRRECH research farm (Table 1).

**Table 1.** Physico-chemical characteristics of Soil.

Soil Type	pH	Depth (cm)	C Organic	N	Clay (%)	Loam	Sand	EC (ds/m)	P (ppm)	K
Clay loam	7/5	0–30	0/66	0.066	5/5	34	60/5	0/75	27.6	400

Pots were watered every other day, to prevent water stress, and maintained in the glasshouse at  $24 \pm 4$  and  $10 \pm 4$  °C (day/night), with an average relative humidity of 60% and approximately 14 h day length. The resultant wheat plants were thinned to 10 seedlings per pot, after emergence (see Figure 2).



**Figure 2.** Glasshouse experiment. Inside the glasshouse, each pot was filled with 4 kg of soil, as described in Table 1, and 10 seedlings were planted in each pot. Watering of the pots took place every other day, and the ambient air temperature was maintained at  $24 \pm 4$  and  $10 \pm 4$  °C (day/night) with a relative humidity of 60% and a day duration of approximately 14 h. (A) Randomizing and (B) uniformly filling pots with soil, (C) preparing pots to measure traits and (D) separating samples based on treatments.

#### 2.3.1. Seed Disinfection

Seed disinfection was performed using the fungicide difenoconazole (Mahan Co. IRI) at 1/1000 (*v/v*) concentration based upon the manufacturer's instructions. Seeds were tested under two disinfection treatments: I, disinfection; and II, no-disinfection.

#### 2.3.2. Sampling and Measurement

For the measurement of plant dry weight, the shoot and root were excised and oven-dried at 80 °C for 48 h, then weighted. Seedling length, root length, dry weight of seedlings, dry weight of root, speed of seed germination, and percentage of seed germination were all measured and recorded.



### Speed and Percentage of Seed Germination

Seed germination (%) and root emergence were determined after 1, 3, 5, 7, and 10 days of incubation. The speed of germination (Equation (1)), seed germination percentage (Equation (2)), and vigor index (Equation (3)) were calculated using the following equations (ISTA, 1985):

$$SG = \frac{\sum ni}{\sum di} \quad (1)$$

$$GP = 100 \times \frac{G}{N} \quad (2)$$

“SG” is the speed of seed germination, “ni” is the number of germinated seeds, “di” is total number of days, “GP” is germination percentage, “G” is the number of germinated seeds during the test, and “N” is the total seeds.

### Vigor Index

Vigor index evaluation was obtained from the final germination percentage (germination percentage on the last day)  $\times$  Seedling length (Equation (3)).

$$VI = (Ls \times Gp)/100 \quad (3)$$

“VI” is the vigor index, “Ls” is the average seedling length (total seedling and root), and “Gp” is germination percentage.

### 2.4. Statistical Analysis

All measurements were conducted in five replications. Data were subjected to the PROC GLM procedure for ANOVA in SAS (SAS 9.2, SAS Institute, SAS/STAT User's Guide, Version 9.2, SAS Inst., Cary, NC, USA, 2009.). Means were separated using the LSD ( $p < 0.05$ ).

## 3. Results

### 3.1. Seed Germination

The results showed that seedling length was significantly affected by cultivar, seed size, and disinfection (Table 2).

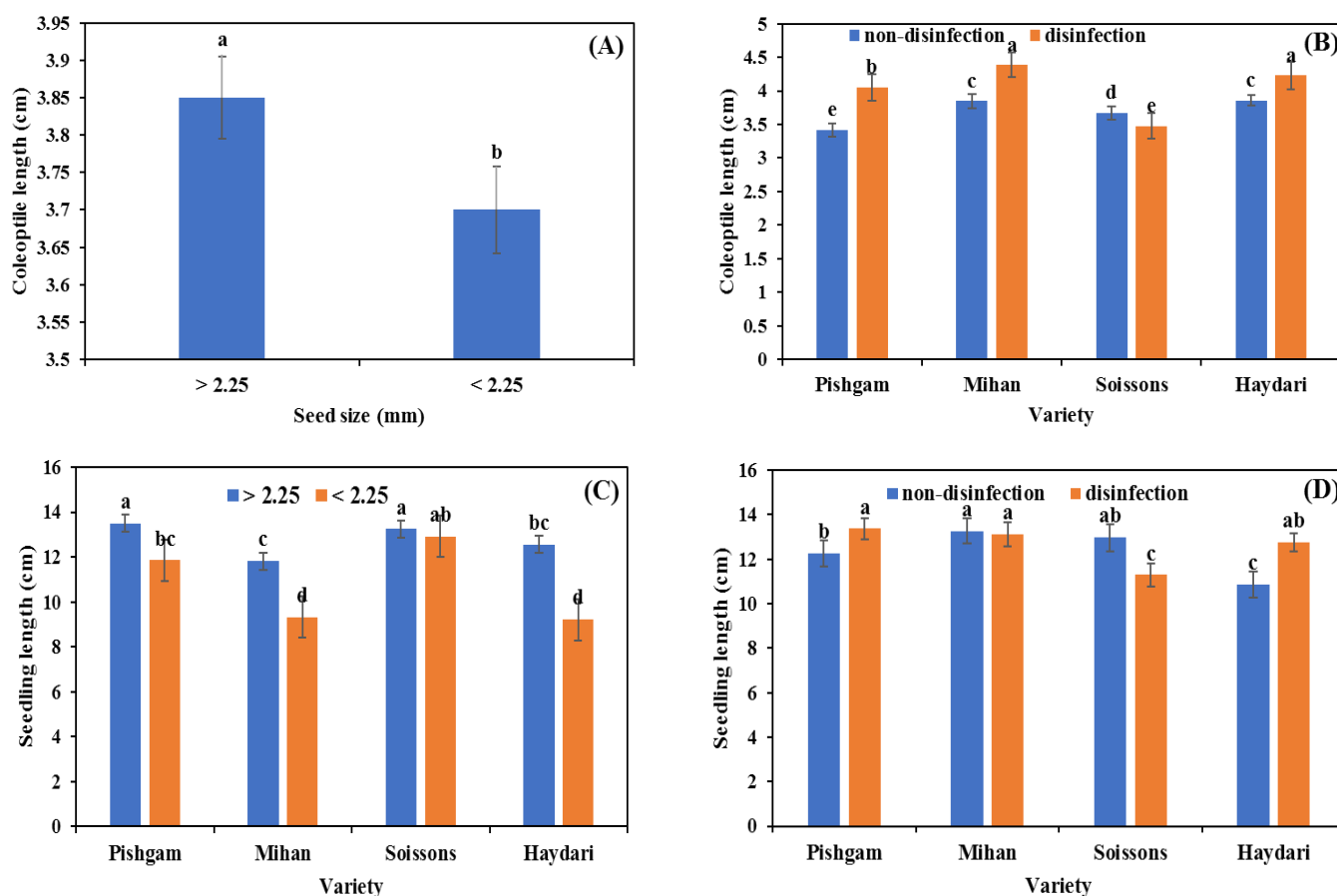
**Table 2.** Effect of cultivar, seed size, and seed disinfection on wheat germination traits.

Treatments	df	MS					
		CL	SL	DW	PG	SG	VI
Cultivars	3	0.77 **	5 **	0.0042 <sup>ns</sup>	0.058 <sup>ns</sup>	0.38 <sup>ns</sup>	5 **
Seed Size	1	0.27 **	0.14 <sup>ns</sup>	0.24 **	0.75 <sup>ns</sup>	0.08 <sup>ns</sup>	0.028 <sup>ns</sup>
Disinfection	1	1.41 **	0.89 <sup>ns</sup>	0.008 *	0.004 <sup>ns</sup>	0.002 <sup>ns</sup>	0.82 <sup>ns</sup>
Cultivars $\times$ Seed Size	3	0.042 <sup>ns</sup>	5.48 **	0.00058 <sup>ns</sup>	6.13 <sup>ns</sup>	0.25 <sup>ns</sup>	3.83 **
Cultivars $\times$ Disinfection	3	0.39 **	7.43 **	0.0002 <sup>ns</sup>	11.5 *	0.27 <sup>ns</sup>	6.89 <sup>ns</sup>
Seed Size $\times$ Disinfection	1	0.076 <sup>ns</sup>	0.01 <sup>ns</sup>	0.0013 <sup>ns</sup>	8.33 <sup>ns</sup>	0.083 <sup>ns</sup>	1.57 <sup>ns</sup>
Cultivars $\times$ Seed Size $\times$ Disinfection	3	0.042 <sup>ns</sup>	0.46 <sup>ns</sup>	0.002 <sup>ns</sup>	6.5 <sup>ns</sup>	0.58 *	0.52 <sup>ns</sup>
Error	32	0.021	0.49	0.018	3.25	0.18	0.46
CV	-	5.86	3.71	1.95	7.16	5.61	3.88

Mean of squares (MS); Coefficient Variation (CV); coleoptile length (CL); seedling length (SL); dry weight (DW); percentage of germination (PG); speed of germination (SG); vigor index (VI). \*, \*\*, and <sup>ns</sup> significant at 5 and 1% probability levels and non-significant, respectively (LSD test;  $p < 0.05$ ).

The largest seedlings were observed in the Pishgam cultivar. The observed differences in coleoptile length may be due to differences in genotype [41]. Seeds larger than 2.25 mm produced the longest seedlings ( $3/85 \pm 0/29$  cm) (Figure 3A). The results also showed that the longest seedling length, about  $4.29 \pm 0.14$  cm, was obtained in the Miham and

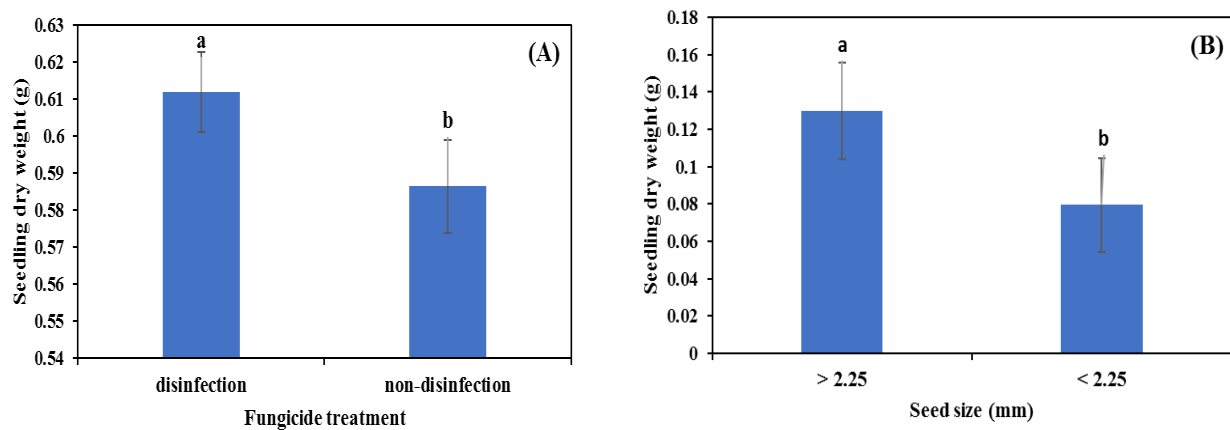
Haydari cultivars, and the seeds that were not treated with disinfectant produced the shortest seedling length ( $3.33 \pm 0.07$  cm) and were obtained using the Pishgam cultivar (Figure 3B).



**Figure 3.** Effect of seed size on coleoptile length (A), seed disinfection on coleoptile length (B), seed size on seedling length (C), and seed disinfection on seedling length in different cultivars (D). The columns' letters indicate the differences between the different treatments ( $p < 0.05$ ) using the LSD test.

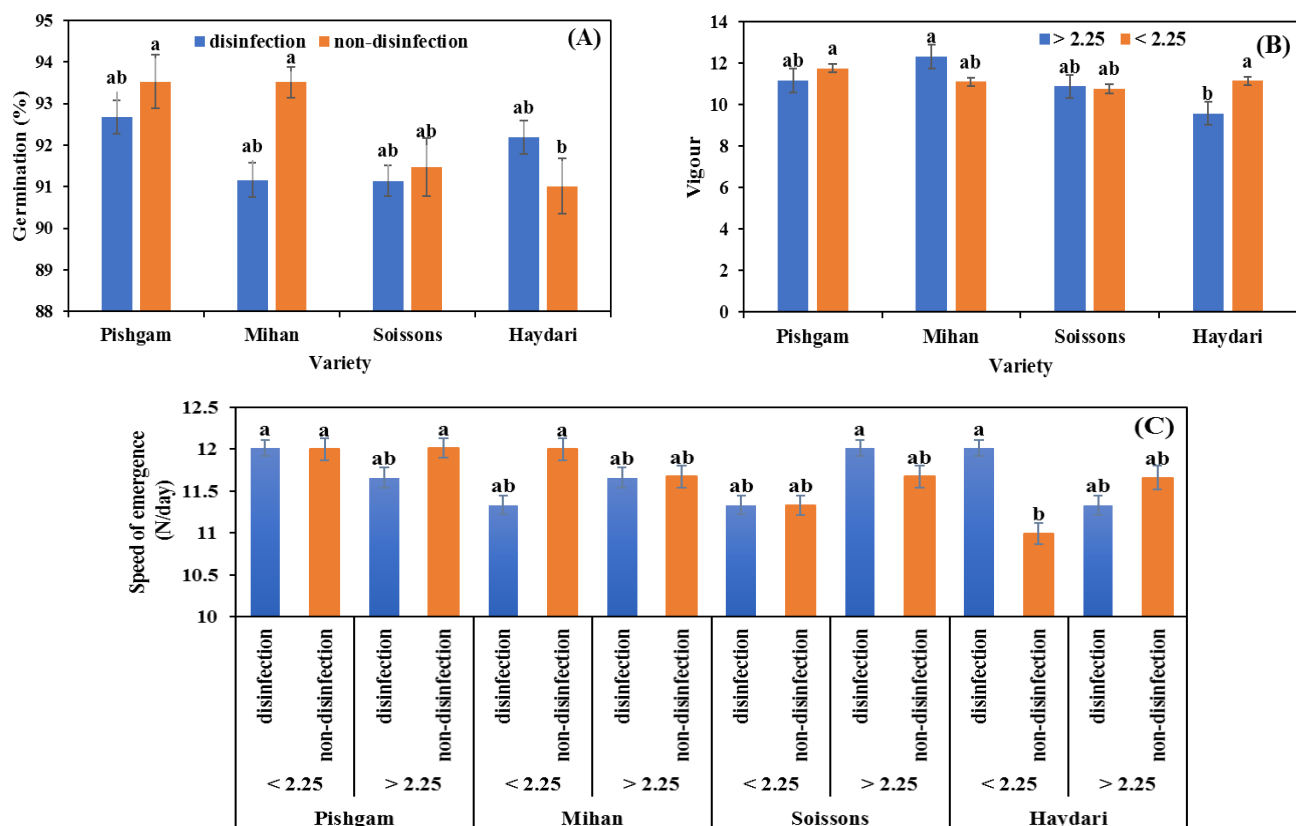
The highest seedling length (ca.  $13.94 \pm 0.22$  cm) was observed in the Pishgam and Soissons cultivars, from seeds larger than 2.25 mm. In contrast, the lowest seedling length ( $10.95 \pm 1.02$  cm) was observed in the Haydari and Mihan cultivars from seeds smaller than 2.25 mm (Figure 3C). The highest seedling length ( $13.42 \pm 0.88$  cm) was observed in the Pishgam and Mihan cultivars in the seeds that were not disinfected. The shortest length ( $10.92 \pm 1.11$  cm) was recorded in plants grown from the Haydari cultivar with seeds that had been disinfected (Figure 3D).

The seedling dry weight was also significantly affected ( $p < 0.01$ ) by seed size and seed disinfection (Table 2). The highest seedling dry weight was  $0.61 \pm 0.08$  g, in seed that had been disinfected and was lowest ( $0.58 \pm 0.08$  g), in seed that had not been disinfected (Figure 4A). The results also indicated that the highest dry weight of seedlings was obtained from seeds larger than 2.25 mm, in all the selected cultivars. Seedling dry weight for the Pishgam, Mihan, Soissons and Haydari cultivars was  $0.68 \pm 0.05$  g,  $0.65 \pm 0.04$  g,  $0.68 \pm 0.02$  g, and  $0.67 \pm 0.03$  g, respectively. The lowest value for the seedling dry weight ( $0.48 \pm 0.03$  g) was obtained from the Pishgam cultivar with seeds smaller than 2.25 mm (Figure 4B).



**Figure 4.** Effect of seed disinfection (A), seed size (B) on dry weight of seedlings. The columns' letters indicate the differences between the different treatments ( $p < 0.05$ ) using the LSD test.

The highest germination percentage ( $93.5 \pm 0.76\%$ ) was observed in the Pishgam and Mihan cultivars without seed disinfection, and the lowest germination percentage ( $91.0 \pm 0.82\%$ ) was obtained in the Haydari cultivar without disinfection (Figure 5A). The highest value for the vigor index ( $12.83 \pm 0.56$ ) was obtained using the Mihan cultivar seeds that were larger than 2.25 mm (Figure 5B). These data also illustrated that the highest germination rate (12 seeds/day) was obtained in the Pishgam cultivar using large seeds. The lowest rate of germination (11 seeds/day) was obtained in the Haydari cultivar, using small seeds, without disinfection (Figure 5C).



**Figure 5.** Effect of seed disinfection on GP (A), seed size on VI (B), and seed disinfection, seed size on SG (C) in different cultivars. The columns' letters indicate the differences between the different treatments ( $p < 0.05$ ) using the LSD test.

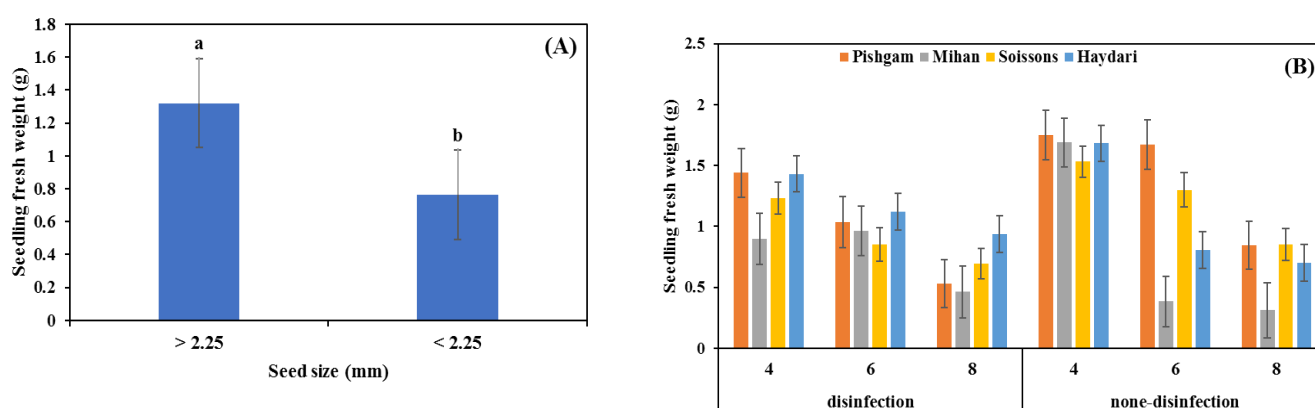
### 3.2. Glasshouse Experiment

Dry weight, fresh weight, and seedling length were all shown to be affected by seed size, disinfection, and sowing depth, in all cultivars (Table 3). The highest seedling fresh weight ( $1.32 \pm 0.61$  g) was measured in large seeds, and the lowest value ( $0.77 \pm 0.47$  g) was measured in small seeds (Figure 6A). In addition, the highest seedling fresh weight ( $1.77 \pm 0.54$  g) was recorded in the Pishgam cultivar using disinfected seeds, at a sowing depth of 4 cm, and the lowest amount ( $0.32 \pm 0.26$  g) was observed using seeds of the Mihan cultivar, without disinfection with seed sown at a depth of 8 cm (Figure 6B).

**Table 3.** Effect of cultivar, seed size, depth sowing, and seed disinfection on wheat germination characteristics.

Treatments	df	MS					
		FW	SL	DW	PG	SG	VI
Cultivars	3	1.24 **	59.24 **	0.13 **	4001.28 **	0.402 **	62.31 **
Seed Size	1	10.76 **	135.91 **	0.01 **	5861.81 **	0.59 **	258.91 **
Disinfection	1	0.95 **	2.58 <sup>ns</sup>	0.011 <sup>ns</sup>	1445.58 **	0.14 **	35.18 **
Sowing depth	2	7.76 **	229.36 **	0.0002 **	13480.9 **	1.35 **	518.7 **
Cultivars × Size	3	0.48 *	15.06 **	0.066 *	243.32 <sup>ns</sup>	0.024 <sup>ns</sup>	2.72 <sup>ns</sup>
Cultivars × Disinfection	3	0.53 *	0.21 <sup>ns</sup>	0.0052 *	74.77 <sup>ns</sup>	0.007 <sup>ns</sup>	1.15 <sup>ns</sup>
Cultivars × Depth	6	0.21 <sup>ns</sup>	25.79 **	0.0044 <sup>ns</sup>	867.6 **	0.085 **	9.26 **
Size × Disinfection	1	0.001 <sup>ns</sup>	0.05 <sup>ns</sup>	0.0025 <sup>ns</sup>	13.29 <sup>ns</sup>	0.001 <sup>ns</sup>	0.26 <sup>ns</sup>
Size × Depth	2	0.19 <sup>ns</sup>	4.28 <sup>ns</sup>	0.0029 <sup>ns</sup>	376.79 *	0.039 *	12.76 **
Disinfection × Depth	2	0.57 *	0.02 <sup>ns</sup>	0.0062 <sup>ns</sup>	62.12 <sup>ns</sup>	0.006 <sup>ns</sup>	2.3 <sup>ns</sup>
Cultivars × Size × Disinfection	3	0.19 <sup>ns</sup>	0.094 <sup>ns</sup>	0.0015 <sup>ns</sup>	343.87 *	0.035 *	6.43 *
Cultivars × Disinfection × Depth	6	0.45 **	0.21 <sup>ns</sup>	0.0044 *	129.03 <sup>ns</sup>	0.012 <sup>ns</sup>	2.28 <sup>ns</sup>
Cultivars × Size × Depth	6	0.10 <sup>ns</sup>	2.5 <sup>ns</sup>	0.00086 <sup>ns</sup>	310.60 *	0.030 **	2.2 <sup>ns</sup>
Size × Disinfection × Depth	2	0.004 <sup>ns</sup>	0.19 <sup>ns</sup>	0.00085 <sup>ns</sup>	19.80 <sup>ns</sup>	0.002 <sup>ns</sup>	0.42 <sup>ns</sup>
Cultivars × Size × Disinfection × Depth	12	0.15 <sup>ns</sup>	1.3 <sup>ns</sup>	0.0014 <sup>ns</sup>	335.37 **	0.033 **	3.53 *
Error	96	0.13	1.68	0.0014	104.98	0.01	1.64
CV	-	14.74	10.66	15.86	16.13	16.05	15.84

Mean of squares (MS); Coefficient Variation (CV); coleoptile length (CL); seedling length (SL); dry weight (DW); percentage of germination (PG); speed of germination (SG); vigor index (VI). \*, \*\*, and <sup>ns</sup> significant at 5 and 1% probability levels and non-significant, respectively (LSD test;  $p < 0.05$ ).

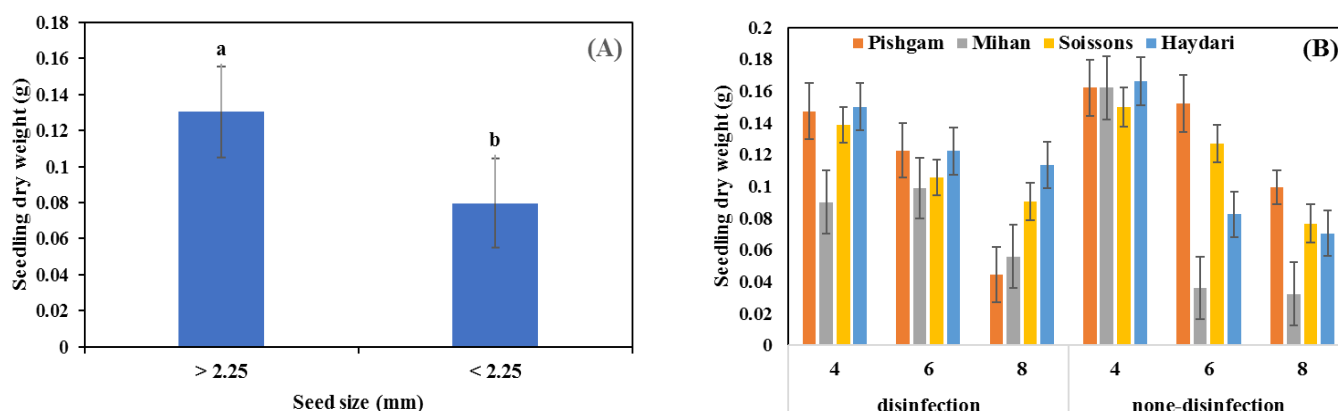


**Figure 6.** Effect of seed size (A), disinfection, cultivars, and depth sowing (B) on seedling fresh weight. The columns' letters indicate the differences between the different treatments ( $p < 0.05$ ) using the LSD test.

The results also showed that the highest dry weight value of the seedling was  $0.13 \pm 0.06$  g, using large seeds, and the lowest dry weight value was  $0.07 \pm 0.04$  g, using the smaller seeds (Figure 7A). The results also demonstrated that the highest seedling dry weight ( $0.167 \pm 0.02$  g) was observed in the Haydari cultivar without disinfection,

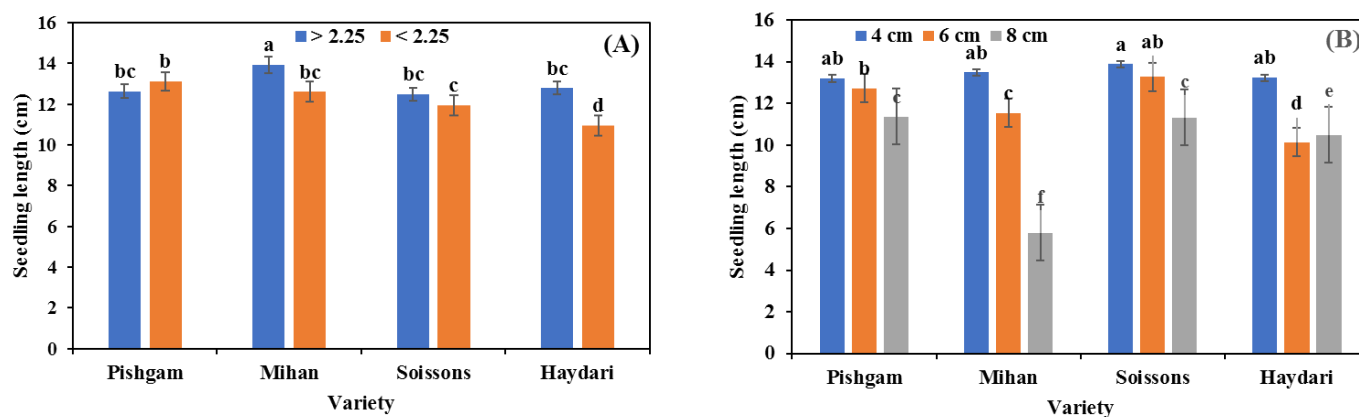


with seeds sown at 4 cm depth, and the lowest was approximately  $0.032 \pm 0.03$  g in non-disinfected seeds of the Mihan cultivar, sown at a depth of 8 cm (Figure 7B).



**Figure 7.** Effect of seed size (A), disinfection, cultivars, and depth of sowing (B) on seedling dry weight. The columns' letters indicate the differences between the different treatments ( $p < 0.05$ ) using the LSD test.

The highest seedling length ( $14.1 \pm 0.92$  cm) was obtained in the Pishgam and Soissons cultivars, using large seeds, and the lowest seedling length ( $9.57 \pm 2.5$  cm) was obtained in the Haydari and Mihan cultivars, using small seeds (Figure 8A). Furthermore, the results of comparisons of mean values related to the interaction of cultivar and sowing depth showed that the highest seedling length ( $14.62 \pm 0.56$  cm) was in the Soissons cultivar, at 4 cm depth, and the lowest seedling length ( $9.6 \pm 0.38$  cm) was also obtained in the seeds of the Mihan cultivar soon, at a depth of 8 cm (Figure 8B).



**Figure 8.** Effect of seed size (A), cultivar and depth of sowing (B) on seedling length. The columns' letters indicate the differences between the different treatments ( $p < 0.05$ ) using the LSD test.

The percentage and speed of germination, and vigor index, were all affected by seed size, disinfection, and depth of sowing, in all cultivars (Table 3). The highest values for germination percentage ( $93.75 \pm 5.1\%$ ), speed of germination ( $0.94 \pm 0.05$  seeds per day), and vigor index ( $13.78 \pm 0.68$ ), were obtained in non-disinfected seeds of the Pishgam cultivar at the shallower sowing depth (4 cm), using large seeds (Table 4). The lowest germination percentage, speed of seed germination, and vigor index were obtained at the 8 cm depth, with seed disinfection, using the smaller size seeds (Table 4).

**Table 4.** Effect of depth of sowing, disinfection, and seed size upon the percentage of germination, speed of seed germination, and vigor index in different cultivars.

Cultivars	Depth	Disinfection	Size (mm)	PG	SG	VI
				(%)	(N/Day)	
PHM	4	Dis	>2.25	79.1 ± 2.94 <sup>a-g*</sup>	0.79 ± 0.02 <sup>a-i</sup>	11.2 ± 0.03 <sup>c-f</sup>
			<2.25	66.7 ± 2.94 <sup>f-k</sup>	0.67 ± 0.02 <sup>f-m</sup>	9.5 ± 0.1 <sup>f-k</sup>
		None-Dis	>2.25	93.7 ± 5.1 <sup>a</sup>	0.94 ± 0.05 <sup>a</sup>	13.7 ± 0.6 <sup>a</sup>
			<2.25	43.7 ± 5.1 <sup>m-q</sup>	0.44 ± 0.05 <sup>o-q</sup>	5.5 ± 0.62 <sup>p-t</sup>
	6	Dis	>2.25	83.3 ± 5.8 <sup>a-e</sup>	0.84 ± 0.05 <sup>a-e</sup>	12.1 ± 0.64 <sup>a-e</sup>
			<2.25	45.8 ± 10.6 <sup>i-p</sup>	0.46 ± 0.1 <sup>n-q</sup>	5.9 ± 1.5 <sup>p-t</sup>
		None-Dis	>2.25	81.2 ± 10.2 <sup>a-f</sup>	0.81 ± 0.1 <sup>a-g</sup>	10.5 ± 0.6 <sup>e-i</sup>
			<2.25	52 ± 2.9 <sup>k-o</sup>	0.52 ± 0.02 <sup>m-p</sup>	6.2 ± 0.1 <sup>o-s</sup>
	8	Dis	>2.25	66.6 ± 7.7 <sup>h-k</sup>	0.67 ± 0.07 <sup>f-m</sup>	8.9 ± 1.2 <sup>h-m</sup>
			<2.25	14.5 ± 2.9 <sup>s</sup>	0.15 ± 0.02 <sup>s</sup>	1.5 ± 0.1 <sup>w</sup>
		None-Dis	>2.25	62.5 ± 10.2 <sup>h-k</sup>	0.63 ± 0.1 <sup>i-m</sup>	7.6 ± 0.5 <sup>j-o</sup>
			<2.25	35.4 ± 4.7 <sup>p-q</sup>	0.35 ± 0.14 <sup>q</sup>	4 ± 1.1 <sup>t-v</sup>
MHN	4	Dis	>2.25	70.83 ± 2.9 <sup>e-i</sup>	0.71 ± 0.02 <sup>d-i</sup>	10 ± 1 <sup>f-i</sup>
			<2.25	66.7 ± 2.9 <sup>h-k</sup>	0.67 ± 0.02 <sup>f-m</sup>	9.3 ± 0.6 <sup>f-k</sup>
		None-Dis	>2.25	87.5 ± 5.1 <sup>a-c</sup>	0.88 ± 0.05 <sup>a-c</sup>	13.3 ± 1.1 <sup>a-c</sup>
			<2.25	16.6 ± 2.8 <sup>s</sup>	0.17 ± 0.12 <sup>s</sup>	1.3 ± 1 <sup>w</sup>
	6	Dis	>2.25	68.7 ± 5.1 <sup>e-i</sup>	0.69 ± 0.05 <sup>e-i</sup>	9.1 ± 0.8 <sup>g-m</sup>
			<2.25	37.5 ± 8.8 <sup>o-q</sup>	0.38 ± 0.08 <sup>pq</sup>	3.2 ± 1 <sup>u-w</sup>
		None-Dis	>2.25	58.3 ± 5.8 <sup>j-m</sup>	0.59 ± 0.05 <sup>k-o</sup>	7.7 ± 1.2 <sup>j-o</sup>
			<2.25	33.3 ± 2.5 <sup>p-r</sup>	0.33 ± 0.23 <sup>qr</sup>	4.13 ± 0.9 <sup>t-u</sup>
	8	Dis	>2.25	70.8 ± 7.7 <sup>d-i</sup>	0.71 ± 0.07 <sup>d-k</sup>	9.4 ± 0.2 <sup>f-k</sup>
			<2.25	29.1 ± 2.1 <sup>q-s</sup>	0.29 ± 0.02 <sup>q-s</sup>	1.9 ± 0.1 <sup>vw</sup>
		None-Dis	>2.25	43.7 ± 4.2 <sup>m-q</sup>	0.44 ± 0.1 <sup>o-q</sup>	5 ± 1.7 <sup>s-u</sup>
			<2.25	18.7 ± 1.5 <sup>r-s</sup>	0.19 ± 0.13 <sup>rs</sup>	1.3 ± 0.9 <sup>w</sup>
SSN	4	Dis	>2.25	89.5 ± 2.9 <sup>ab</sup>	0.9 ± 0.02 <sup>ab</sup>	13.1 ± 0.1 <sup>a-c</sup>
			<2.25	79.1 ± 5.8 <sup>a-g</sup>	0.79 ± 0.05 <sup>a-h</sup>	11.3 ± 0.9 <sup>b-e</sup>
		None-Dis	>2.25	91.6 ± 5.8 <sup>a</sup>	0.92 ± 0.05 <sup>ab</sup>	13.7 ± 1.3 <sup>a</sup>
			<2.25	64.5 ± 2.9 <sup>g-k</sup>	0.65 ± 0.02 <sup>h-m</sup>	7.4 ± 0.2 <sup>i-p</sup>
	6	Dis	>2.25	68.7 ± 5.1 <sup>g-k</sup>	0.69 ± 0.05 <sup>e-i</sup>	9.9 ± 0.8 <sup>f-i</sup>
			<2.25	62.5 ± 5.1 <sup>h-k</sup>	0.63 ± 0.05 <sup>i-m</sup>	7.3 ± 0.8 <sup>m-q</sup>
		None-Dis	>2.25	68.7 ± 5.1 <sup>e-i</sup>	0.69 ± 0.05 <sup>e-i</sup>	9.8 ± 0.4 <sup>f-k</sup>
			<2.25	54.1 ± 2.9 <sup>j-n</sup>	0.54 ± 0.02 <sup>l-p</sup>	7.1 ± 0.6 <sup>j-o</sup>
	8	Dis	>2.25	78.5 ± 10 <sup>abc</sup>	0.87 ± 0.1 <sup>a-c</sup>	12.6 ± 1.4 <sup>a-d</sup>
			<2.25	41.6 ± 2.9 <sup>n-q</sup>	0.42 ± 0.02 <sup>pq</sup>	5 ± 0.5 <sup>s-u</sup>
		None-Dis	>2.25	70.82 ± 7.7 <sup>d-i</sup>	0.71 ± 0.07 <sup>d-j</sup>	9.6 ± 1.4 <sup>f-j</sup>
			<2.25	52 ± 11.7 <sup>k-o</sup>	0.52 ± 0.11 <sup>m-p</sup>	6.4 ± 1.5 <sup>o-s</sup>
HDI	4	Dis	>2.25	91.6 ± 2.9 <sup>ab</sup>	0.92 ± 0.02 <sup>ab</sup>	13.4 ± 0.6 <sup>ab</sup>
			<2.25	70.8 ± 7.7 <sup>d-i</sup>	0.71 ± 0.07 <sup>d-k</sup>	9 ± 1.1 <sup>g-m</sup>
		None-Dis	>2.25	89.5 ± 5.8 <sup>ab</sup>	0.9 ± 0.05 <sup>ab</sup>	13.3 ± 1 <sup>a-c</sup>
			<2.25	64.5 ± 5.8 <sup>g-k</sup>	0.65 ± 0.05 <sup>d-k</sup>	7.2 ± 1.2 <sup>m-q</sup>
	6	Dis	>2.25	81.2 ± 8.8 <sup>a-f</sup>	0.82 ± 0.08 <sup>a-f</sup>	10.8 ± 1.6 <sup>d-h</sup>
			<2.25	62.5 ± 6.8 <sup>h-k</sup>	0.62 ± 0.08 <sup>j-m</sup>	6.8 ± 2 <sup>n-s</sup>
		None-Dis	>2.25	66.6 ± 5.8 <sup>f-j</sup>	0.68 ± 0.05 <sup>f-i</sup>	8.7 ± 0.7 <sup>i-n</sup>
			<2.25	66.6 ± 5.8 <sup>f-j</sup>	0.66 ± 0.05 <sup>f-i</sup>	5.5 ± 0.5 <sup>p-t</sup>
	8	Dis	>2.25	85.42 ± 7.2 <sup>a-d</sup>	0.68 ± 0.04 <sup>a-d</sup>	11.07 ± 0.7 <sup>d-g</sup>
			<2.25	77 ± 2.9 <sup>b-h</sup>	0.77 ± 0.02 <sup>b-j</sup>	5.5 ± 0.5 <sup>p-t</sup>
		None-Dis	>2.25	72.9 ± 7.7 <sup>c-i</sup>	0.73 ± 0.07 <sup>c-k</sup>	5.1 ± 0.4 <sup>q-u</sup>
			<2.25	60.4 ± 2.9 <sup>i-l</sup>	0.61 ± 0.02 <sup>j-n</sup>	5.2 ± 0.3 <sup>r-u</sup>
LSD (5%)				16.6	0.16	2.08

PHM (Pishgam); MHN (Mihan); SSN (Soissons); HDI (Haydari); PG (percentage of germination); SG (speed of germination); VI (vigor index). \* Mean of values ± standard deviation of the respective traits. Values followed by different letters are significantly different according to an LSD test at  $p < 0.05$ .

## 4. Discussion

### 4.1. Seed Size

The data reported have revealed the importance of seed size in the germination and growth and development of wheat plants in all the cultivars examined. Seeds larger than 2.25 mm showed a greater germination capacity, even in the presence of disinfection. As is well known, seed germination requires a large amount of energy, which is provided by the oxidation of the endosperm [42]. The seed must provide sufficient nutrients for seedling growth, because the seedling is dependent on the seed for adequate growth [17,43]. The results shown in Table 3 demonstrate that the seed size significantly influences the coleoptile length, the seedling length, the dry weight, the percentage of germination, the speed of germination and the vigor index. At the same time, the effect of the cultivar together with the size of the seed only show significant differences in the growth of the seedling, but not in the germination phases. Moreover, it seems that the cultivars do not differ in the germinative response, but rather in the subsequent stages of growth. In general, seeds retain all the genetic information inherited from the mother plant necessary for germination and growth [2]. At the same time, unfavorable environmental conditions can block germination [44]. In the current study, disinfection can be considered a stressful condition that can inhibit the germination processes of wheat seeds. Hence, larger seeds with developed endosperm are more likely to germinate and develop better. Indeed, large seeds are known to produce larger wheat seedlings, which most probably leads to better seedling establishment and increased yield productivity in the field [45]. According to previous studies, seed size is also positively correlated with vigor, and accordingly, larger seeds tend to produce stronger seedlings [46]. Once the germination process has started, the grain begins to develop coleoptile and other more complex structures. However, there are a number of other factors that reduce the growth of coleoptiles and thereby increase the risk of failure in seedling establishment [46]. For example, there is evidence that a change in the diameter of the coleoptile may be related to changes in embryo size. Our data confirm that wheat seeds with a diameter of less than 2.25 mm develop and grow more slowly. This supports the relationship between seed size and coleoptile diameter, with larger seeds also having larger embryos, leading to the production of larger-diameter coleoptiles that have the ability to grow better and more strongly even when grown in poor soil conditions [17,47].

### 4.2. Seed Disinfection

In the current study, seed disinfection was shown to have an inhibitory effect upon coleoptile growth in the Pishgam, Mihan, and Haydari cultivars, which may be related to the chemical composition of the materials used in the seed disinfectant. Another point to note is that the Soissons cultivar displayed a positive reaction to the use of fungicide for disinfection. There are several recent reports on the effect of seed disinfection on the vigor of seedling growth in the literature [48–51]. A study in 2019 showed that, although the fungicides procymidone and iprodione triadimenol are effective in protecting seedlings, they also possess phytotoxic effects, leading to a delay in seed germination and a reduction in the developmental speed of *Allium cepa* seedlings [52]. In addition to using high-quality seeds, the use of fungicides to achieve high yields is a widespread agricultural practice [53–56]. The chemical treatment of seeds is one of the most effective methods of controlling both fungi and insects [37]. However, Leslie and colleagues found that in wheat [57], and Rajjou and collaborators in studies of soybean [58], that some chemical treatments, when left on the seeds for a long time, may reduce seed germination and prevent seedling survival. According to Ayesha's research, the seedling phytotoxicity effect of fungicides depends upon the type of fungicide used, and the storage time of the disinfected seeds [59]. In addition, the cytogenotoxicity effects of these chemical compounds were shown to cause chromosomal damage and cell death in meristematic cells [52]. Conversely, it has also been reported that some seeds treated with fungicides had higher germination rates than the control treatment [60]. The majority view in the

literature is that chemical treatment of seeds has a negative effect on plant development [61]. When used in the seed treatment of wheat, the Triazole group of fungicides can cause cell toxicity, reduce mesocotyl length, and produce gaps in leaf tips, that prevent seedling emergence from the soil [62]. Abati's results showed that treatment of wheat grains with the fungicide triadimenol increased the development of abnormal seedlings and the appearance of seedlings with twisted, thick, and wide leaves [63]. In addition, Radzikowska and colleagues observed that seed treatment with triadimenol resulted in lower values for seedling length [64]. These data suggest that this fungicide may have a phytotoxic effect on wheat seedlings, as a result of which the rate of seedling emergence in the field will be reduced [65].

As mentioned previously, different cultivars respond differently to different chemical products, which is consistent with our results [65]. Several studies have shown that increasing the rate and percentage germination of disinfected cotton seeds, may be due to the reduction of seed contamination and the stimulatory effect of germination in seed which has been disinfected. However, Sivachandiran and colleagues observed a decrease in the percentage and rate of germination in cotton seeds treated with high concentrations of fungicide and insecticide [66].

#### 4.3. Sowing Depth

Our data have demonstrated that a greater sowing depth delays both the germination and growth processes of the wheat cultivars studied. In fact, plants sown at a depth of 8 cm have a significantly lower percentage and speed of germination and vigor index than those grown at a depth of 4 cm. Reducing in seed germination due to increasing planting depth has been proven in numerous studies [67,68]. Wheat plants that appear later in the field have less biomass and produce fewer spikes, which reduces the final yield. In Australia [42,65] and other countries [34,69], lower wheat yields have been reported to occur when seeds are sown too deep. Surveys of Australian farmers' have shown that, when wheat with short coleoptiles is planted at depths of more than 5 cm, grain yield is reduced by at least 10% [70]. According to Bazzaz, the optimal sowing depth for planting of wheat is 4 cm [71]. Studies have also demonstrated a direct association between coleoptile length and seedling emergence at different planting depths [34]. When seeds were treated with sowing depths of 2.4 and 4.1 inches, with tebuconazole fungicide, seedling emergence was slower than other treatments, which is consistent with the results of our study. The fungicide tebuconazole has been reported to reduce germination when compared to untreated seeds at a depth of 4.1 inches. Seedlings treated with tebuconazole appear to grow more slowly than other treatments, especially when planted more than 3 inches deep [64]. Enilconazole systemic fungicides have also been shown to inhibit seedling emergence and establishment in the field [72].

#### 5. Conclusions

The results have shown that the highest percentage and speed of seed germination, as well as highest vigor index, were obtained at a seed sowing depth of 4 cm in non-disinfected wheat seeds, larger than 2.25 mm, using the Pishgam cultivar. Larger seeds contain larger nutrient stores, which thereby improves their seed germination indices, (germination percentage and speed of germination) followed by subsequent increases in coleoptile length, seedling length, seedling dry weight, and overall seed vigor. These data also illustrated that seed disinfection in the Pishgam and Haydari cultivars had inhibitory effects on coleoptile growth and seedling length, which could be related to the fungicide's chemical composition. Overall, crop quality and quantity decrease when using poor quality small seed, with the application of inappropriate agrochemicals, and with seed sown at a suboptimal sowing depth. Bearing in mind the global importance of wheat in relation to food security, the results presented represent an important preliminary investigation of the effect of seed size, sowing depth, and disinfection on germination characteristics

and seedling growth of wheat cultivars and may assist and inform future field studies of these phenomena.

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