agriculture

MDPI

Article

Effect of Osmopriming with Melatonin on Germination, Vigor and Health of *Daucus carota* L. Seeds

Agnieszka Rosińska *, Roman Andrzejak and Vignan Kakkerla

Department of Phytopathology, Seed Science and Technology, Poznań University of Life Sciences, Dąbrowskiego 159, 60-594 Poznań, Poland; roman.andrzejak@up.poznan.pl (R.A.)
* Correspondence: agnieszka.rosinska@up.poznan.pl

Abstract: Carrot is one of the most frequently grown vegetables in Poland and in the world. Seed-borne pathogenic fungi negatively influence their quality as well as the quantity and quality of carrot root yield. Melatonin is a PGR, which includes protective effects against biotic and abiotic stress factors and antioxidant effects. The aim of this experiment was to determine the effect of osmopriming with melatonin on germination, vigor and carrot seeds health. Carrot seeds were osmoprimed in a solution of polyethylene glycol (PEG) with an osmotic potential of −1.5 MPa at 20 °C for 7 days; melatonin was added to the PEG solution at doses of 25, 50, 100 or 200 µM. Generally, osmopriming with the addition of melatonin significantly improved germination capacity at first and final counts (sample I about 7–14% and sample II 35–43%), reduced the incidence of *Alternaria alternata*, *A. radicina* and *Fusarium* spp. and increased the percentage of non-sporulating hyphae. Treating accelerated the germination of seeds at a significant rate in comparison with untreated seeds and treated with fungicide, especially at low dosage, i.e., 25–50 µM. MGT of primed seeds with the addition of melatonin at dose 25 µM shortened about 0.5 day (sample I) and 1 day (sample II) The effect of melatonin on seed quality parameters was comparable or better than treating with fungicide. The results suggest that melatonin could replace fungicides in the future, which are harmful to the environment.

Keywords: carrot; seed priming; germination; seed vigor; *N*-acetyl-5-methoxytryptamine; parasitic fungi

Citation: Rosińska, A.; Andrzejak, R.; Kakkerla, V. Effect of Osmopriming with Melatonin on Germination, Vigor and Health of *Daucus carota* L. Seeds. *Agriculture* **2023**, *13*, 749. https://doi.org/10.3390/agriculture13040749

Academic Editor: Jose C. Jimenez-Lopez

Received: 27 February 2023
Revised: 16 March 2023
Accepted: 22 March 2023
Published: 23 March 2023

1. Introduction

Carrot (*Daucus carota* L.) seeds are exposed on pathogenic microorganisms infection during seed production, harvesting, cleaning, transport and their storage. Seed-borne pathogens negatively influence on seed germination, decrease seed vigor and shorten the seed storability [1]. Richardson [2] listed the most important seed-borne pathogens, which include: *Alternaria dauci* (J.G. Kuhn) J.W. Groves & Skolko, *Alternaria radicina* Mei-er, Dreschler & E.D. Eddy, *Cercospora carotae* (Pass.) Kazn. & Siem., *Eryshipe heraclei* DC, *Sclerotinia sclerotiorum* (Lib.) de Bary and *Xanthomonas hortorum* pv. *carotae* (Kendrick) Vauterin, Hoste & Swings. Alternaria Black Rot of carrots, caused *by Alternaria radicina*, is a fungi disease that affects carrots. Infected roots are characterized by black, sunken lesions. They mostly affect carrots that are kept in cold storage, where they can quickly spread [3]. Additionally, *A. radicina* reduced seed germination capacity and seedlings survival. This fungus is capable of producing phytotoxins radicin (RAD), radicinol (ROH) and epi-radicinol (epi-ROH), which are phytotixic to carrot seedlings [4]. Chen et al. [5], among the mechanisms of phytotoxins, listed protein targeting, damaging the membrane structure and disintegrating the cytoskeleton. All of these mechanisms providing to plant cell apoptosis. Similarly, Alternaria Leaf Blight is a foliar disease, caused by *Alternaria dauci* and *Cercospora carotea* [6]. *Alternaria dauci* occurs on older leaves, while *C. carotea* spots occur on young leaves [7]. They are mostly causing yield loss by reducing leaf photosynthetic

area and also breaking the carrot petioles [8]. The production of secondary metabolites by *A. dauci* plays a key role in the fungus-plant interaction and determines the aggressiveness of this fungus [9]. *Alternaria dauci* produces numerous non-host selective (NHST) and host-selective (HST) toxins. The disease symptoms, as spots, on carrot leaves are the result of the effect of one or several toxins [10]. Some mycotoxins, such as tenuazonic acid, alternariol and alternariol monomethyl ether, induce hydrogen peroxide accumulation in leaves, which was proven by Meena et al. [11] in tomato leaves. Zinniol disintegrates the cell membrane due to its effect on calcium channels [12].

Osmopriming has been shown to be an effective method to improve the most important seed quality parameters, such as germination and vigor, by increasing speed and uniformity of germination [13]. The priming of seeds in high osmotic conditions has been described as a convenient way to overcome drought and salinity problems [14]. The osmopriming process is referred to as the time-dependent soaking of seeds in low potential inorganic salts or polyethene glycol (PEG) necessary for optimum water absorption that enhances a better seed performance [15]. Bradford [13] and Miladinov et al. [16] opined that this technique consists of seed hydration in a solution whose osmotic potential is enough to allow initial germination to occur but not adequate for radicle protrusion. Priming of carrots seeds increased seed vigor and yield, and thus, resulting in greater incomes compared to other forms of treatment [17–19]. Aazami and Zahedi [14] reported that priming had a positive influence on carrot germination-related traits, and concurrently, decrease abnormal seedlings. Polyethylene glycol (PEG) of 8000 molecular weight is commonly used for seed priming. However, a side effect, increased fungal colonization and penetration into deeper seed tissues, has been observed after priming in PEG solution [20–23]. Tylkowska and van den Bulk [23] claimed that this increase was greater in seeds with medium to high initial levels of infection. Therefore, the seed health, undergoing this treatment, is very important. In order to counteract the growth of microorganisms during priming, various chemical and natural substances are used. Dorna et al. [24] studied the effect of priming China aster seeds in PEG 8000 solution and in combination with seed treatment with the fungicide Rovral 50 WP (a.i. iprodoone). The application of the fungicide before, during and after priming lowered the incidence of fungi on the seeds compared to control seeds and those treated with PEG alone.

Melatonin (*N*-acetyl-5-methoxytryptamine) is tagged as a multi-regulatory molecule because of its abundant biological actions in plants such as the ability to act as a plant biostimulator against stress, both abiotic and biotic. In addition, it can regulate plant growth, regulate vegetative development process in plants such as leaf senescence, rooting, photosynthetic efficiency, and biomass yield, is involved in regulation of circadian rhythms and photoperiodic reactions and also plays a vital role in regulating the processes of flowering and the formation and ripening of fruits and seeds [25–29]. Furthermore, the protection of plants under drought, high temperature, cold, copper, lead, salinity and pathogen infection by melatonin has been affirmed by researchers [30–35]. Additionally, melatonin has been described as an important antioxidant that has actively acted against a variety of toxic oxygen and nitrogen in plants [36,37]. In seeds, melatonin plays a vital role by providing antioxidative defense in a relatively dry system that cannot be regulated. Kołodziejczyk et al. [38] confirmed that melatonin stimulates activity of antioxidant enzymes, i.e., superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione peroxidase (GSH-PX). Additionally, it is an essential compound that protects seeds' reproductive tissues from harmful environmental conditions [39,40]. Xiao et al. [41] examined the impact of melatonin on *Gossypium hirsutum* seed germination, and reported that a low concentration of melatonin can promote *Gossypium hirsutum* seed germination by increasing the activity of antioxidant enzymes and thereby enlarging the accumulation of the malondialdehyde (MDA), and plant growth regulators (PGRs) are regulated. In addition, exogenous melatonin was observed to improve antioxidant defense in *Cucumis sativus* seeds germinated under chilling stress by Bałabusta et al. [42], who confirmed that melatonin could provide *C. sativus* seeds and young seedlings against oxidative stress both directly and indirectly

detoxifying reactive oxygen species (ROS), thus making the plant grown better in more harsh and harmful environmental conditions.

The aim of the study was to evaluate the protective influence of melatonin during seed treatment, priming, under conditions favoring the development of pathogenic fungi that reduce germination and emergence of *D. carota* 'Amsterdamska' seeds. The phenomenon of seed health deterioration during osmopriming treatment is common, and it negatively affects seed quality. Melatonin, as a plant growth regulator, can improve germination by preventing excessive growth of fungi, especially pathogenic fungi, i.e., *Alternaria alternata*, *A. dauci*, *A. radicina* and *Fusarium* spp. The research hypothesis is to improve, through the use of osmopriming with melatonin, the most important quality parameters, assessed prior to seed market release, germination, health and also vigor.

2. Materials and Methods

2.1. Materials

In the experiment, two seed lots of carrot (*Daucus carota* L.) 'Amsterdamska', i.e., PL004/63/51/612A (sample I) and PL804/63/51/414A (sample II), were used. Both seed lots were obtained from a Polish seed company.

Seeds were treated with melatonin. Melatonin is soluble in organic solvents, such as ethanol or dimethyl formamide. The solubility of melatonin in ethanol (C_2H_5OH) is approximately 50 mg·mL^{-1}.

Seeds were treated with contact fungicide Zaprawa nasienna T (a. s. thiuram), produced by Synthos Agro—chemical control.

2.2. Methods

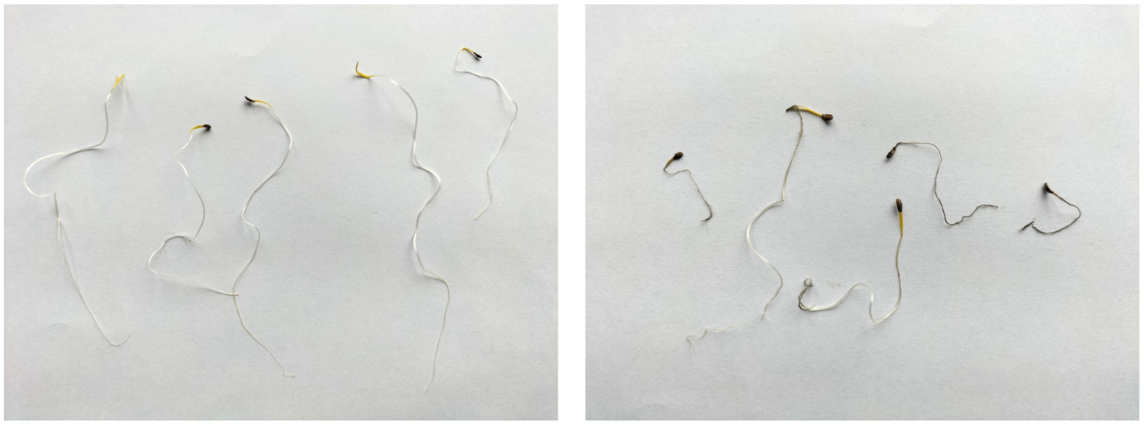
For osmopriming, 50 seeds (800 seeds were treated for each concentration) of *D. carota* 'Amsterdamska' were placed in 9 cm diameter sterile Petri dishes on four layers of sterile blotter moistened with polyethylene glycol 8000 solution (PEG) with the addition of melatonin at a concentration of 25, 50, 100 and 200 μM. To each Petri dish, 5 mL of PEG solution with melatonin was added. Before melatonin was added to the PEG solution, it was dissolved in ethanol (50 mg of melatonin in 1 mL of ethanol). The concentration of PEG used was 355 g·L^{-1} water to give a nominal osmotic potential at 20 °C of −1.5 MPa [43]. Then, Petri dishes with osmoprimed seeds were closed with parafilm and incubated for seven days without light at 20 °C. After seven days, primed seeds, from each combination, were washed separately using a tap water for 5 min and next rinsed three times in sterile water to remove PEG. Subsequently, the seeds were surface dried on sterile blotter, then placed in semi-open Petri dishes and dried at 20 °C and 45% relative humidity for 48 h to achieve an equilibrium moisture content.

2.3. Seed Germination Test

A seed germination test was conducted according to the recommendations of the International Seed Testing Association (ISTA) [44]. Seeds were placed in Petri dishes on six layers of blotter paper moistened with 5 mL of distilled water (Figure 1). For each treatment, 300 seeds were used, on six repeats of 50 seeds. Seeds were incubated at 20 °C in darkness.

Figure 1. Seeds of both samples prepared for the germination test.

After 7 days of incubation, seed germination at the first count was determined, whereas after 14 days, germination at the final count was evaluated. Moreover, after 14 days of incubation, the percentage of diseased and deformed seedlings and the percentages of fresh and dead seeds were determined (Figure 2).



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

Figure 2. Normal seedlings (**a**) and abnormal diseased seedlings (**b**) after 14 days of incubation.

2.4. Seed Vigor Test

In order to determine the seed vigor, the germination speed and uniformity were assessed, and the following parameters were evaluated: T_1, T_{25} (time required to germinate 1 and 25% of the total percentage of germinating seeds—Gmax), MGT (mean germination time) and $U_{75\text{-}25}$ (time between 25 and 75% of Gmax). Seed vigor was assessed under the same conditions as during seed germination. For the test, 300 seeds from each treatment were used. Seeds were placed in Petri dishes on six layers of blotter paper moistened with 5 mL of distilled water. Germinated seeds were counted every day, until no new germinating seeds occurred, and were removed from Petri dishes. The seeds were considered as germinating when the radicle was at least 1 mm long.

2.5. Seed Health Test

The deep-freeze blotter test for seed health assessment was used. For each treatment method, 200 seeds were tested. In total, 20 seeds were placed in each Petri dish. Seeds were incubated for 3 days in darkness at 20 °C, and were then incubated for 24 h at −20 °C. After freezing, seeds were incubated under alternating cycle of 12 of NUV light and 12 h of darkness for 6 days at 20 °C. Identification of fungi were evaluated using a stereo microscope. The basis for identification of the fungi was their growth and sporulation color [45–47]. The percentages of seeds infested with individual fungi and seeds free of fungi were calculated (Figure 3).

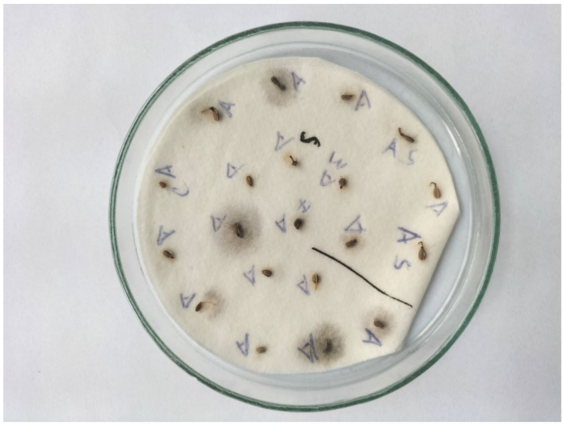


Figure 3. Petri dish with carrot seeds after examination of seed health.

Parameters characterizing seed vigor T_1, T_{25}, MGT and $U_{75\text{-}25}$ were evaluated using SeedCalculator 2.1. [48]. All results were evaluated using Statistica, by a one-way analysis of variance after transforming percentage values according to Bliss' formula: y = arc sin [sqr (x/100)]. Means were compared with the Duncan's multiple range test at the level $\alpha = 0.05$.

3. Results

Seed Germination Test

Germination capacity at first and final counts of untreated carrot seeds of sample I were 79.0% and 79.7%, respectively. Control seeds were characterized also with the highest percentage of abnormal diseased seedlings and dead seeds. After osmopriming with the addition of melatonin at a concentration of 50, 100 and 200 µM and treating with a fungicide, germination at first and final counts were significantly increased in comparison to untreated seeds (Table 1). Germination capacity of untreated seeds of sample II was lower than sample I. Germination at first count was 46.3% and after the next 7 days did not change. Osmopriming with melatonin significantly improved these parameters. Osmoprimed seeds with the addition of melatonin at a concentration of 50 µM at first and final counts germinated to the same extent as the fungicide application.

Untreated seeds of both analyzed seed samples characterized by the highest percentage of abnormal diseased seedlings, especially seeds of sample II—31%. In case of sample II, the low germination capacity was an effect of the high percentage of abnormal diseased seedlings and dead seeds (17%). Osmopriming with melatonin significantly lowered the number of diseased seedlings of both samples. After treating seeds with fungicide, the lowest number of abnormal diseased seedlings of sample II was observed. However, osmopriming with melatonin also significantly reduced number of diseased seedlings, regardless of the dose from 19.7 to 26%. There was no effect of seed treatment with a fungicide and osmopriming with melatonin on the percentage of fresh seeds and abnormal deformed seedlings in comparison with untreated seeds. Significantly lower dead seeds of sample I compared with the control seeds were observed after osmopriming with melatonin

at a concentration of 100 and 200 µM. In the case of sample II, all methods of osmopriming significantly reduced the percentage of dead seeds as the same level as fungicide treatment (Table 1).

Table 1. Effects of osmopriming with the addition of melatonin on the seed germination of *D. carota* 'Amsterdamska' seeds (%).

Seed Treatment	Germination Capacity				Abnormal Diseased Seedlings		Abnormal Deformed Seedlings		Fresh Seeds		Dead Seeds	
	At First Count		At Final Count									
Sample I												
U *	79.0	a	79.7	a	5.7	b	0.7	a	2.0	ab	12.0	c
F	92.0	c	94.0	c	1.0	a	0	a	0	a	5.0	a–c
OS25	86.3	b	87.7	b	2.3	a	0	a	2.3	b	7.7	bc
OS50	91.7	bc	92.3	bc	1.3	a	0	a	1.3	ab	5.0	a–c
OS100	89.3	bc	94.3	c	1.0	a	0	a	2.0	ab	2.7	a
OS200	89.7	bc	91.7	bc	1.0	a	0	a	4.3	b	3.0	ab
Sample II												
U	46.3	a	46.3	a	31.0	d	0	a	5.7	a	17.0	b
F	87.7	cd	93.7	d	0.7	a	1.0	a	2.7	a	2.0	a
OS25	82.3	bc	83.0	b	11.3	c	0.3	a	1.3	a	4.0	a
OS50	89.0	d	90.0	cd	5.0	b	0.7	a	2.0	a	2.3	a
OS100	81.7	b	85.3	bc	6.3	b	0	a	6.0	a	2.3	a
OS200	83.0	b-d	86.0	bc	9.0	bc	0	a	1.7	a	3.3	a

* U—untreated seeds (control), F—fungicide, OS25—osmoprimed seeds with the addition of melatonin 25 µM, OS50—osmoprimed seeds with the addition of melatonin 50 µM, OS100—osmoprimed seeds with the addition of melatonin 100 µM, OS200—osmoprimed seeds with the addition of melatonin 200 µM. Means in the columns followed by the same letter are not significantly different at $\alpha = 0.05$ level.

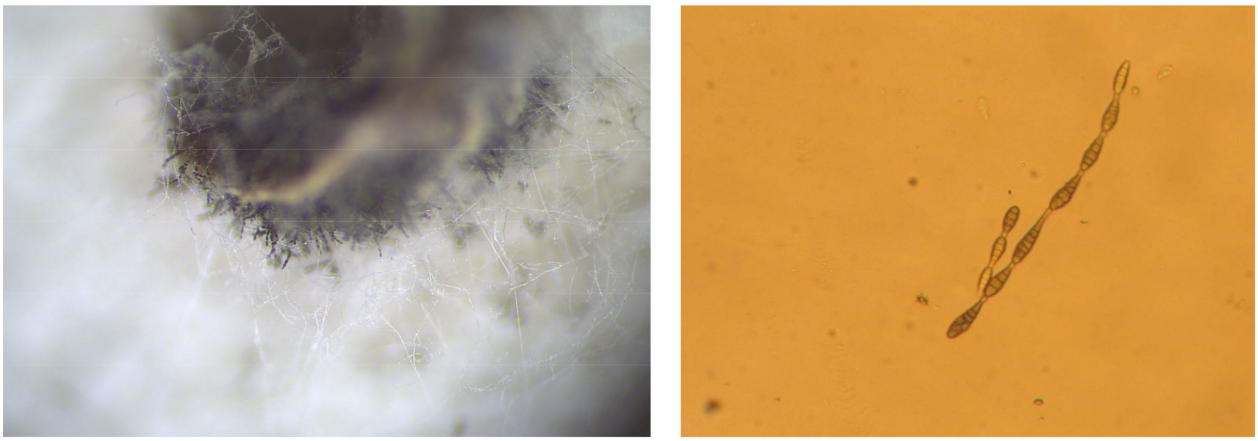
Seeds of both samples osmoprimed with melatonin at a concentration of 25 and 50 µM germinated the fastest. All parameters which describe the speed of germination (T_1, T_{25} and MGT) were significantly lower in comparison with untreated seeds and seeds treated with a fungicide. In case of sample II, seeds osmoprimed with the addition of melatonin at a concentration of 100 and 200 µM also germinated faster than untreated and parameters T_{25} and MGT were significantly lower. Osmopriming with melatonin did not affect the uniformity of germination (U_{75-25}) (Table 2).

Table 2. Effects of osmopriming with the addition of melatonin on the speed and uniformity of *D. carota* 'Amsterdamska' seed germination (days).

Seed Treatment	T_1 **		T_{25}		MGT		U_{75-25}	
Sample I								
U *	1.4	c	1.93	b	2.44	b	0.71	a
F	1.11	b	1.95	b	2.52	b	0.99	a
OS25	0.45	a	1.33	a	1.90	a	1.06	a
OS50	0.48	a	1.40	a	1.97	a	1.06	a
OS100	1.31	bc	1.94	b	2.53	b	0.88	a
OS200	1.07	b	1.90	b	2.46	b	0.97	a
Sample II								
U *	1.94	d	2.59	d	3.13	d	0.83	a
F	2.00	d	2.68	d	3.34	e	0.95	a
OS25	0.99	a	1.75	a	2.23	a	0.85	a
OS50	1.49	b	2.07	b	2.50	b	0.70	a
OS100	1.77	cd	2.31	c	2.81	c	0.72	a
OS200	1.68	c	2.33	c	2.85	c	0.81	a

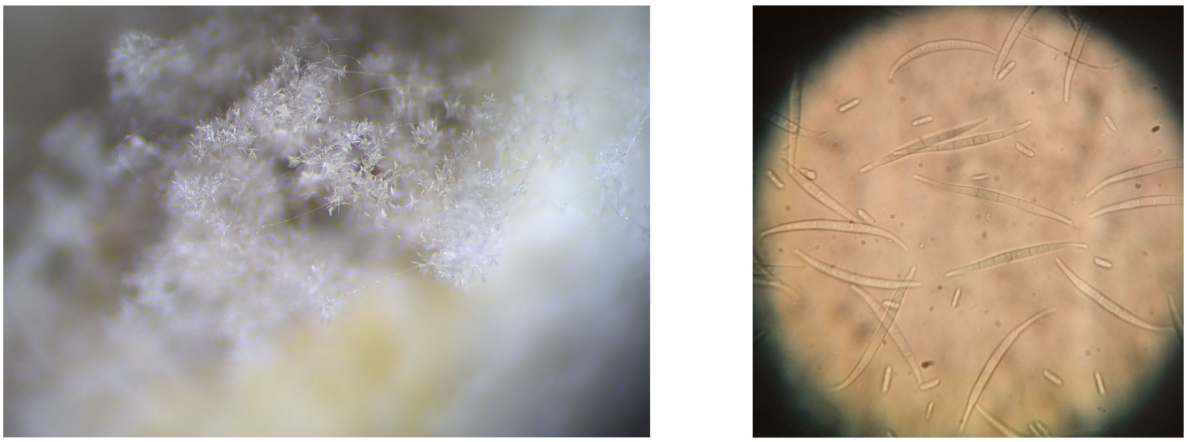
* for explanations, see Table 1. ** T_1—time to 1% of Gmax (the percentage of germinating seeds), T_{25}—time to 25% of Gmax, MGT—mean germination time, U_{75-25}—time between 25 and 75% of Gmax. Means in columns followed by the same letter are not significantly different at $\alpha = 0.05$ level.

Untreated seeds of sample I were occupied by the following fungi *Alternaria alternata* (Fr.) Keissl. (Figure 4), *Cladosporium* spp., *Fusarium* spp. (Figure 5), *Melanospora simplex* (Corda) D. Hawksw. and *Stemphylium botryosum* Wallr. Among them, the most frequent *A. alternata* (80.5%), *Cladosporium* spp. (50.5%) and *M. simplex* (50%) were found. The fungicide application reduced the incidence of all fungi and increased the percentage of seeds free of fungi. Osmopriming with melatonin was not as effective as fungicide treatment; however, reduction of *A. alternata*, *Cladosporium* spp. and *Fusarium* spp. was noted. After osmoprimed with the addition of 25 µM melatonin, 29.5% less seeds were occupied by *A. alternata* in comparison with untreated seeds. Adding, during osmopriming, melatonin at a concentration of 25–100 µM reduced incidence of *Cladosporium* spp. by about 10–26.5% in relation to untreated seeds. Osmopriming with melatonin at a concentration of 100 and 200 µM decreased the percentage of seeds infected by fungi of genera *Fusarium* by 24.5 and 23.5%, respectively. There was no effect of osmopriming on seed colonization by *Melanospora simplex* and *Stemphylium botryosum* and the percentage of seeds free of fungi. A significant increase in the number of seeds with non-sporulating hyphae was observed after seed priming with melatonin, compared to untreated and fungicide treated seeds, particularly after the addition of melatonin at a concentration of 50 and 100 µM (Table 3).



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

Figure 4. *Alternaria alternata* on carrot seeds (**a**) and spores (**b**).



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

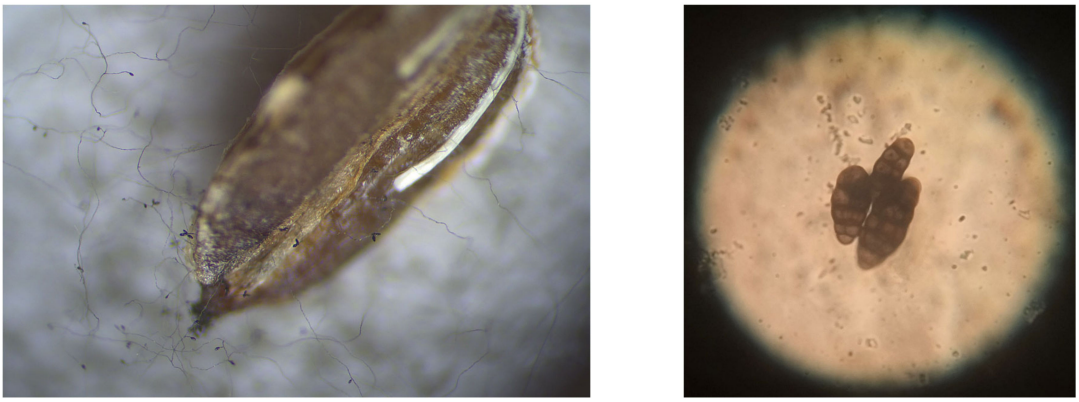
Figure 5. *Fusarium* spp. on carrot seeds (**a**) and spores (**b**).

Table 3. Effects of osmopriming with the addition of melatonin on the incidence of fungi on *D. carota* 'Amsterdamska' seeds of sample I and seeds free of fungi (%).

Seed Treatment	*Alternaria alternata*		*Cladosporium* spp.		*Fusarium* spp.		*Melanospora simplex*		*Stemphylium botryosum*		Non-Sporulating Hyphae		Seeds Free of Fungi	
U *	80.5	d	50.5	c	36.5	e	50.0	b	18.5	ab	11.0	a	1.0	a
F	16.5	a	6.0	a	4.0	a	0.5	a	10.0	a	10.5	a	59.0	b
OS25	51.0	b	37.0	b	20.5	cd	46.5	b	24.5	b	30.5	c	2.5	a
OS50	75.0	cd	40.5	b	22.0	d	42.0	b	27.5	b	50.0	d	1.5	a
OS100	65.0	bc	34.0	b	12.0	b	41.5	b	27.5	b	41.5	d	2.5	a
OS200	61.5	bc	41.5	bc	13.0	bc	39.0	b	24.0	b	19.0	b	0	a

* for explanations, see Table 1. Means in columns followed by the same letter are not significantly different at $\alpha = 0.05$ level.

Untreated seeds of sample II were highly infested with *Alternaria alternata* (97%). In addition, these seeds were settled by *Alternaria radicina* (25%) (Figure 6), *Cladosporium* spp. (29.5%), *Fusarium* spp. (8.5%), *Melanospora simplex* (28%) and *Stemphylium botryosum* (12%). Treating by fungicide decreased the incidence of fungi to the greatest extent. Osmopriming with melatonin, irrespective of the applied dose, reduced the occurrence of *A. radicina* and *Fusarium* spp. to the same level as the fungicide. No osmopriming method reduced the incidence of the fungi of the genus *Cladosporium* and *Stemphylium botryosum* on the seeds. The addition of melatonin at a concentration of 50 and 200 µM significantly increased seed settlement by *Melanospora simplex* compared to untreated seeds. The highest number of seeds with non-sporulating hyphae was observed after osmopriming with melatonin at a concentration of 50 µM (44.5%). After adding melatonin at a concentration of 25 and 100 µM, significantly more seeds with non-sporulating hyphae in comparison with untreated seeds were also found. Significantly more seeds free of fungi were observed only after seed treatment with a fungicide—58.5%—particularly after the addition of melatonin at a concentration of 50 and 100 µM (Table 4).



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

Figure 6. *Alternaria radicina* on carrot seeds (**a**) and spores (**b**).

Table 4. Effects of osmopriming with the addition of melatonin on the incidence of fungi on *D. carota* 'Amsterdamska' seeds of sample II and seeds free of fungi (%).

Seed Treatment	*Alternaria alternata*		*Alternaria radicina*		*Cladosporium* spp.		*Fusarium* spp.		*Melanospora simplex*		*Stemphylium botryosum*		Non-Sporulating Hyphae		Seeds Free of Fungi	
U *	97.0	d	25.0	b	29.5	b	8.5	b	28.0	b	12.0	bc	4.0	a	0	a
F	27.5	a	3.0	a	3.5	a	1.5	a	0	a	6.0	a	7.0	ab	58.5	b
OS25	84.0	c	13.5	a	31.0	b	2.5	a	33.5	bc	18.0	c	18.0	c	0	a
OS50	69.5	b	9.0	a	30.0	b	1.5	a	38.0	c	17.5	c	44.5	d	0.5	a
OS100	70.0	b	4.5	a	30.0	b	3.0	a	32.0	bc	13.5	bc	11.0	bc	0	a
OS200	80.0	c	8.0	a	33.5	b	4.0	ab	39.5	c	8.5	ab	9.5	a-c	0	a

* for explanations, see Table 1. Means in columns followed by the same letter are not significantly different at $\alpha = 0.05$ level.

4. Discussion

Melatonin is a plant growth regulator (PGR), which is involved in plant growth, development and inactivation of free radicals. It was detected for the first time in 1995 in nine edible plants; currently, we know of its presence in over about 300 plants, which produce endogenous melatonin in various parts, such as roots, seeds, etc. [41]. The presence of endogenous melatonin in *D. carota* roots was confirmed by Hattori et al. [49]. The precursor of melatonin in plant cells, as in animals and humans, is tryptophan. The use of molecular techniques has allowed the determination of the melatonin biosynthetic pathway in plants, and it is known that it can take place by several mechanisms [50]. However, scientists are constantly studying the contribution of melatonin to physiological processes in plants and its possible applications. According to Manchester et al. [39], melatonin in seeds may be crucial in protecting germ and reproductive tissues of plants from oxidative damage due to abiotic and biotic stresses. The present work is focused on the possibility of improving seed quality parameters, such as germination, vigor and seed health, by adding it during the osmopriming.

The complex biomedical and physiological process of seed germination is controlled by PGRs, such as organic acids: abscisic acid (ABA), salicylic acid (SA), gibberellins (GAs) and polyamines (PAs) [51]. The exogenous application of these agents with osmoprimed seeds has been observed to play an important and effective role in the seed germination process [52]. Previously, positive effects of pre-sowing treatment with melatonin on seed germination of *Cucumis sativus*, *Phacelia tanacetifolia*, *Carthamus tinctorius*, *Gossypium hirsutum*, *Brassica oleracea rubrum* and *Zea mays* var. *ceratina* were found [41,53–56]. Jiang et al. [57] reported that *Zea mays* seeds priming with melatonin at 0.8 mmol L^{-1} significantly improved germination energy, germination percentage, proline content and total phenolic content, decreasing membrane relative electrolyte leakage and lipid peroxidation product. The authors added that priming with melatonin under salt stress conditions also had a positive effect on seedling growth. According to Murch and Erland [50], who analyzed the last 20 years of research under melatonin in plants, this PGR stimulates lateral and adventitious root formation by the interaction with auxins. Moreover, melatonin interacts with other phytohormones involved in root development, such as ABA or cytokinins. High levels of melatonin during germination were strongly correlated with better seedling performance, especially under abiotic and biotic stresses. In this study, was observed that osmoprimed *D. carota* seeds with the addition of melatonin germinated faster, their germination capacity at first and final counts were higher and they were characterized by a lower percentage of abnormal seedlings and dead seeds in comparison with control seeds. These results demonstrated that the influence of osmopriming with melatonin on *D. carota* 'Amsterdamska' seeds germination promote their germination, especially of sample II. After osmopriming seeds of sample II with the addition of melatonin at a dose of 50 µM, the germination at first and final counts were higher, i.e., about 42.7 and 43.7%, respectively. It should be mentioned that osmopriming, controlled hydration of seeds, is a treatment that improves seed germination and vigor. Enzymes, such as amylases and proteases, responsible for the hydrolysis and mobilization of reserve materials, are synthesized and activated during priming. The result of that treatment is higher, faster and more uniform germination of seeds [58,59]. On the other hand, osmopriming can reduce germination due to a significant increase in seed colonization by fungi that can infect seedlings. The results of this experiment show that despite the presence of fungi on the seeds, the seedlings after osmopriming with melatonin had no disease symptoms. The highest percentage of seedlings with disease symptoms was recorded for untreated seeds, especially for sample II—31%. After osmopriming with melatonin, significantly less diseased seedlings were observed. One of the causes of seedling dieback is the production of mycotoxins by fungi. The protective effect of melatonin against mycotoxins, i.e., aflatoxins, ochratoxin B, fumonisins, HT-2, deoxynivalenol and b-zearanelol, has been confirmed in animal cells [60]. This may indicate a protective effect of melatonin, by activating a series of enzymatic reactions.

Melatonin slows the damage of plant cells and stimulates systemic acquired resistance (SAR) [61].

The addition of PGRs or bioactive compounds, such as GAs, proline or melatonin, can positively affect seed germination and seedlings emergence [62]. Posmyk et al. [54] confirmed that seeds are capable of absorbing melatonin during osmopriming, and noted high levels of melatonin in *Cucumis sativus* seeds after 4 days of priming. It is known that melatonin enhances the resistance of plants to abiotic stresses, such as drought, cold, salt damage and high temperatures, and biotic stresses, such as fungi, bacteria and viruses [41,63–65]. This effect was explained by Xiao et al. [41], who claimed that melatonin promotes the water absorption and accumulation of biomass in seeds. The authors treated cotton seeds with melatonin (10–200 μM) and observed an increase of the fresh weight of the seeds after treatment. Chen et al. [66] observed that the exogenous application of melatonin at a concentration of 100 μM of effectively stimulates root growth of *Brassica juncea* young seedlings. Additionally, Bai et al. [67] claimed that seeds soaked with melatonin at a concentration of 100 μM showed an enhanced seed germinated rate and improved the radical length and fresh weight of seedlings compared with untreated seeds. Melatonin also stimulates the growth of seedlings and aboveground part of plants [38]. *Daucus carota* seed vigor is an important parameter, which affects seedling emergence and plant growth in the field. High vigorous carrot seeds result in increased emergence, plant height and dry matter during the vegetative phase, according to Chipenete et al. [68]. The authors stated that the use of less vigorous seeds might result in yield reductions of up to 27%. A positive effect of PEG-6000 osmopriming with melatonin at a dose of 20 μM was found in *Triticale hexaploide* seeds, as the germination rate of primed seeds was higher, by about 57.7%, than untreated seeds. Additionally, primed seeds were characterized by better gas-exchange, higher relative chlorophyll concentration, enhanced the activities of SOD and POD and decreased reactive oxygen species (ROS) and MDA content in the seeds and seedlings [69]. Hanci [70] observed that *Daucus carota* seeds soaked in solutions with a low concentration of melatonin (1.5, 3 and 4.5 μM) had significantly better vigor indexes at 300 mM salinity conditions than untreated ones. The results of our study show that all applied methods increased seed vigor, i.e., seeds germinated faster. However, melatonin at lower concentrations (25 and 50 μM) had a more beneficial effect on seed vigor than that applied at higher concentrations (100 and 200 μM). Seeds of the sample I, which were osmoprimed with the addition of melatonin at a concentration of 25 μM, had lower values of parameters T_1, T_{25} and MGT than untreated seeds, by 0.95, 0.6 and 0.54 days, respectively. In the case of sample II, these values were lower by about 0.95, 0.84 and 0.9 days. This could be attributed to the apparent inhibitory effect and pro-oxidative action of treatment with a higher concentration of melatonin, as indicated by Simlat et al. [71], whose findings affirm that melatonin at a low concentration (5 and 20 μM) significantly improved the seed vigor and properties of *Stevia rebaudiana* seedlings. Similar results were reported by Posmyk et al. [53], who showed that melatonin at a concentration of 100 μM was toxic to the growth and vigor of *Brassica oleracea rubrum* seedlings as compared with melatonin at low concentrations (1 and 10 μM). Xiao et al. [41] reported that *Gossypium hirsutum* seeds treated with 10–50 μM melatonin germinated faster than seeds without usage of melatonin. The authors noted that melatonin at low dosage (20 μM) reduced the accumulation of malondialdehyde (MDA) and regulated the PGRs, such as GAs and ABA.

The findings from this study show that fungicides can be replaced by osmopriming with melatonin regardless of the dose, as it effectively reduces the percentage of seedlings with disease symptoms, which makes it as effective as the use of fungicide. Additionally, this could be the most auspicious method to enhance ecological crops, protect the environment and support healthy food production. The reduction in the number of diseased seedlings may be related to the protective effect of melatonin against fungal infection. Arnao and Hernandez-Ruiz [27,72] mentioned that melatonin is an effective biocide against fungi and bacteria, but the mechanism of action is not yet well known. Yin et al. [73] applied melatonin to enhance resistance to Marssonina apple blotch (*Diplocarpon mali*)

by *Malus prunifolia* 'Donghongguo'; they explained its protective effect by maintaining intracellular hydrogen peroxide (H_2O_2) concentrations at stable levels and intensifying the activities of plant defense-related enzymes. In studies on the use of melatonin for plant protection against *Phytophtora nicotianae* (Oomycota), growth inhibition, reduced cell viability and inhibition of virulence of this pathogen were found. After the application of melatonin, cytoplasm fragmentation was observed in hyphal cells of *P. nicotianae* in combination with the disappearance of cell organelles. On the other hand, the authors not observe a significant inhibitory effect on hyphal growth of *Alternaria solani*, *Botrytis cinerea*, *Fusarium oxysporum*, *Penicillium expansum* and *Verticillium daliae*. According to the authors, this is the result of different wall cell structures in oomycota and true fungi [74].

Meanwhile, the research results indicate that osmopriming with melatonin can be adopted to reduce the occurrence and improve the tolerance level of *Daucus carota* 'Amsterdamska' seeds to some of the identified fungi pathogens. However, as expected, the most effective treatment in reducing the fungi incidents was the application of fungicide. From the research findings, osmopriming with melatonin irrespective of the dose also significantly reduced the incidence of *Alternaria alternata*, *A. radicina* and *Fusarium* spp. Melatonin had a more significant suppression ability at a higher concentration. This aligns with the findings of Arnao and Hernandez-Ruiz [27] that melatonin at of different concentrations inhibited the growth of several plant fungal pathogens, such as *Alternaria* spp., *Fusarium* spp. and *Botrytis* spp. According to Lee et al. [75], melatonin can induce plant resistance to fungi such as *Fusarium* spp., working as a signal to initiate defense responses, including the activation of defense-related genes and transcription factors. Similar results were obtained by Liu et al. [76], who found that melatonin treatment, especially at a dose of 50 µM, enhances resistance of *Lycopersicon esculentum* fruit against to *Botrytis cinerea* through regulating the H_2O_2 level and JA signaling pathway, which plays an important role in plant pathogen defense. Zhao et al. [77] claimed that resistance against pathogens is connected with sugar metabolism (galactose, cellulose and xylose), and found differential expression of genes during *Arabidopsis thaliana* development that was modulated by melatonin. The cell walls of the plant were reinforced during infection. Moreover, Kobylińska et al. [35] reported that melatonin influences on carbohydrates nutrition during stress conditions in non-photosynthesizing cells, such as seed cells. The authors found a mechanism of redirecting of amino acid carbon skeletons to the production of sugars during the gluconeogenesis. Perhaps, these mechanisms also took place in the cells of *D. carota* 'Amsterdamska' seeds, protecting them from pathogen penetration.

Osmopriming with melatonin at a concentration of 50, 100 and 200 µM significantly increased the colonization of seeds by *Melanospora simplex*, *Mucor* spp., non-sporulating hyphae and after osmopriming with melatonin at a concentration of 100 µM *Ulocladium* spp. This implies that the application of melatonin to *D. carota* 'Amsterdamska' seeds stimulates the growth of these fungi and can reduce the seed quality. Additionally, the reduction in seed colonization by fungi belonging to the genera *Alternaria* and *Fusarium* provided the opportunity for other fungi to develop. The highest percentage of non-sporulating hyphae was observed after osmopriming seeds with melatonin at a concentration of 50 µM. Probably, melatonin delays fungal growth and its sporulation.

5. Conclusions

The research findings demonstrated that the effect of osmopriming with melatonin on *D. carota* 'Amsterdamska' seed germination is closely related to its concentration, and 50–200 µM can be defined as the highest concentration rate to promote carrot seed germination. Additionally, the findings from this study show that the use of fungicide can be substituted by osmopriming with melatonin regardless of the dose, as it effectively reduces the percentage of seedlings with disease symptoms. Employing this tool can reduce the environmental pollution that is generally associated with chemical pesticide applications. This is particularly important for osmoprimed seeds, as the treatment promotes fungal growth on treated seeds, while melatonin has a protective effect against pathogens. Additionally,

the emerging strategies from this research that reveal the importance of osmopriming and melatonin application on important crops such as carrots can be adopted for resistance against diverse fungal pathogens. The key findings of this study can be useful while exploring the melatonin-based alternate approaches for fungi control in carrots and other plant-related seeds. However, further research is needed on the effects of melatonin on seed health and the growth and development of pathogenic fungal mycelium. There are still few data in the literature on this subject and the protective mechanism against fungi is unclear.

Author Contributions: Conceptualization, A.R.; methodology and formal analysis, A.R. and R.A.; investigation and data curation, A.R., R.A. and V.K.; writing—original draft preparation, A.R., R.A. and V.K.; writing—review and editing, A.R. and R.A. All authors have read and agreed to the published version of the manuscript.

Funding: This publication was co-financed within the framework of the Polish Ministry of Science and Higher Education's program: "Regional Excellence Initiative" in the years 2019–2023 (No. 005/RID/2018/19)", financing amount 12 000 000,00 PLN.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Xue, Z.; Wang, R.; Ning, H.; Li, W.; Bai, Y.; Li, Y. Evaluation and management of fungal infected carrot seeds. *Sci. Rep.* **2020**, *10*, 10808. [CrossRef]
2. Richardson, M.J. *An Annotated List of Seed-Borne Diseases*; International Seed Testing Association: Zurich, Switzerland, 1990.
3. Que, F.; Ho, X.L.; Wang, G.L.; Xu, Z.S.; Tan, G.F.; Li, T.; Wang, Y.H.; Khadr, A.; Xiong, A.S. Advances in research on the carrot, an important root vegetable in the Apiaceae family. *Hort. Res.* **2019**, *6*, 69. [CrossRef] [PubMed]
4. Moliszewska, E.; Smiatek, V. Toxic properties of Alternaria radicina culture filtrates against carrot seeds and seedlings. *Ecol. Chem. Eng. A* **2009**, *16*, 1515–1523.
5. Chen, H.; Singh, H.; Bhardwaj, N.; Bhardwaj, S.K.; Khatri, M.; Kim, K.-H.; Peng, W. An exploration on the toxicity mechanisms of phytotoxins and their potential utilities. *Crit. Rev. Environ. Sci. Technol.* **2020**, *52*, 395–435. [CrossRef]
6. Gugino, B.K.; Carroll, J.; Chen, J.; Ludwig, J.; Abawi, G. *Carrot Leaf Blight Diseases and Their Management in New York*; Cornell University Press: New York, NY, USA, 2004.
7. Farrar, J.J.; Pryor, B.A.; Davis, R.M. Alternaria diseases of carrot. *Plant Dis.* **2007**, *88*, 776–784. [CrossRef]
8. Gugino, B.; Carroll, J.; Widmer, T.; Chen, P.; Abawi, G. Field evaluation of carrot cultivars for susceptibility to fungal leaf blight diseases in New York. *Crop Prot.* **2007**, *26*, 709–714. [CrossRef]
9. Courtial, J.; Hamama, L.; Hélesbeux, J.-J.; Lecomte, M.; Renaud, Y.; Guichard, E.; Voisine, L.; Yovanopoulos, C.; Hamon, B.; Ogé, L.L.; et al. Aldaulactone—An original phytotoxic secondary metabolite involved in the aggressiveness of Alternaria dauci on carrot. *Front. Plant Sci.* **2018**, *9*, 502. [CrossRef]
10. Lecomte, M.; Hamama, L.; Voisine, L.; Gatto, J.; Hélesbeux, J.-J.; Séraphin, D.; Peña-Rodriguez, L.M.; Richomme, P.; Boedo, C.; Yovanopoulos, C.; et al. Partial resistance of carrot to Alternaria dauci correlates with in vitro cultured carrot cell resistance to fungal exudates. *PLoS ONE* **2014**, *9*, e101008. [CrossRef]
11. Meena, M.; Zehra, A.; Dubey, M.K.; Aamir, M.; Gupta, V.K.; Upadhyay, R.S. Comparative evaluation of biochemical changes in tomato (*Lycopersicon esculentum* Mill.) infected by Alternaria alternata and its toxic metabolites (TeA, AOH, and AME). *Front. Plant Sci.* **2016**, *7*, 1408. [CrossRef]
12. Barash, I.; Mor, H.; Netzer, D.; Kashman, Y. Production of zinniol by *Alternaria dauci* and its phytotoxic effect on carrot. *Physiol. Plant Pathol.* **1981**, *19*, 7–16. [CrossRef]
13. Bradford, K.J. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *Hort. Sci.* **1986**, *21*, 1105–1112. [CrossRef]
14. Aazami, M.A.; Zahedi, S.M. Germination of carrot (*Daucus carota* L.) seeds in response to osmotic priming. *Thai J. Agri. Sci.* **2018**, *51*, 188–194.
15. Amooaghaie, R. The effect of hydro and osmopriming on alfalfa seed germination and antioxidant defenses under salt stress. *Afr. J. Biotechnol.* **2011**, *10*, 6269–6275. [CrossRef]
16. Miladinov, Z.; Balešević-Tubić, S.; Đukić, V.; Ilić, A.; Čobanović, L.; Dozet, G.; Merkulov-Popadić, L. Effect of priming on soybean seed germination parameters. *Acta Agric. Serb.* **2018**, *23*, 15–26. [CrossRef]
17. Aazami, M.A.; Mohammadi, S. Determination of the best temperature and dry condition in seeds-carrot primed. *Pak. J. Biol. Sci.* **2008**, *11*, 1502–1505. [CrossRef]

18. Draganic, I.; Lekic, S. Seed priming with antioxidants improves sunflower seed germination and seedling growth under unfavorable germination conditions. *Turk. J. Agric. For.* **2012**, *36*, 421–428. [CrossRef]
19. Dellan, E.; Lagunovschi-Luchian, V. Germination and vigour of primed *Daucus carota* L. seeds under saline stress conditions. *Rom. Biotechnol. Lett.* **2015**, *20*, 10833–10840.
20. Tylkowska, K.; Biniek, A. Fungi and germination of carrot and parsley seeds under osmopriming and fungicide treatment. *Phytopatol. Pol.* **1996**, *12*, 51–61.
21. Nascimento, W.M.; West, S.H. Microorganism growth during muskmelon seed priming. *Seed Sci. Technol.* **1998**, *26*, 531–534.
22. Tylkowska, K.; van den Bulk, R.W. Effects of osmo- and hydropraming on fungal infestation levels and germination of carrot (*Daucus carota* L.) seeds contaminated with *Alternaria* spp. *Seed Sci. Technol.* **2001**, *29*, 365–375.
23. Zhao, X.; Li, Y.; Dorna, H. Effect of priming and fungicide treatment on germination of China aster (*Callistephus chinensis* L.) seeds. *Seed Sci. Technol.* **2004**, *32*, 417–424. [CrossRef]
24. Dorna, H.; Tylkowska, K.; Zhao, X. Effects of osmopriming and Rovral on health and germination of China aster seeds. *Phytopatol. Pol.* **2001**, *21*, 35–44.
25. Kolár, J.; Machácková, I. Melatonin in higher plants: Occurrence and possible functions. *J. Pineal Res.* **2005**, *39*, 333–341. [CrossRef] [PubMed]
26. Arnao, M.B.; Hernández-Ruiz, J. Melatonin: Plant growth regulator and/or biostimulator during stress? *Trends Plant Sci.* **2014**, *19*, 789–797. [CrossRef] [PubMed]
27. Arnao, M.B.; Hernández-Ruiz, J. Functions of melatonin in plants: A review. *J. Pineal Res.* **2015**, *59*, 133–150. [CrossRef]
28. Hernández-Ruiz, J.; Arnao, M.B. Phytomelatonin, an interesting tool for agricultural crops. *Focus Sci.* **2016**, *2*, 1–7. [CrossRef]
29. Nawaz, M.A.; Huang, Y.; Bie, Z.; Ahmed, W.; Reiter, R.J.; Niu, M.; Hameed, S. Melatonin: Current status and future perspectives in plant science. *Front. Plant Sci.* **2016**, *6*, 1230. [CrossRef]
30. Li, C.; Wang, P.; Wei, Z.; Liang, D.; Liu, C.; Yin, L.; Jia, D.; Fu, M.; Ma, F. The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*. *J. Pineal Res.* **2012**, *53*, 298–306. [CrossRef]
31. Li, C.; Zhao, Q.; Gao, T.; Wang, H.; Zhang, Z.; Liang, B.; Wei, Z.; Liu, C.; Ma, F. The mitigation effects of exogenous melatonin on replant disease in apple. *J. Pineal Res.* **2018**, *65*, e12523. [CrossRef]
32. Szafrańska, K.; Glińska, S.; Jamas, K.M. Ameliorative effect of melatonin on meristematic cells of chilled and re-warmed *Vigna radiata* roots. *Biol. Plant.* **2013**, *57*, 91–96. [CrossRef]
33. Shi, H.; Tan, D.X.; Reiter, R.J.; Ye, T.; Yang, F.; Chan, Z. Melatonin induces class A1 heat shock factors (HSFA1s) and their possible involvement of thermotolerance in Arabidopsis. *J. Pineal Res.* **2015**, *58*, 335–342. [CrossRef]
34. Ye, J.; Wang, S.; Deng, X.; Yin, L.; Xiong, B.; Wang, X. Melatonin increased maize (*Zea mays*) seedling drought tolerance by alleviating drought-induced photosynthetic inhibition and oxidative damage. *Acta Physiol. Plant.* **2016**, *38*, 48. [CrossRef]
35. Kobylińska, A.; Borek, S.; Posmyk, M. Melatonin redirects carbohydrates metabolism during sugar starvation in plant cells. *J. Pineal Res.* **2018**, *64*, e12466. [CrossRef]
36. Tan, D.X.; Chen, L.D.; Poeggeler, B.; Manchester, L.C.; Reiter, R.J. Melatonin: A potent, endogenous hydroxyl radical scavenger. *Endocr. J.* **1993**, *1*, 57–60.
37. Galano, A.; Tan, D.X.; Reiter, R.J. Melatonin as a natural ally against oxidative stress: A physicochemical examination. *J. Pineal Res.* **2011**, *51*, 1–16. [CrossRef]
38. Kołodziejczyk, I.; Kaźmierczak, A.; Posmyk, M.M. Melatonin application modifies antioxidant defense and induces endoreplication in maize seeds exposed to chilling stress. *Int. J. Mol. Sci.* **2021**, *22*, 8628. [CrossRef]
39. Manchester, L.C.; Tan, D.X.; Reiter, R.J.; Park, W.; Monis, K.; Qi, W. High levels of melatonin in the seeds of edible plants: Possible function in germ tissue protection. *Life Sci.* **2000**, *67*, 3023–3029. [CrossRef]
40. Murch, S.J.; Alan, A.R.; Cao, J.; Saxena, P.K. Melatonin and serotonin in flowers and fruits of *Datura metel* L. *J. Pineal Res.* **2009**, *47*, 277–283. [CrossRef]
41. Xiao, S.; Liu, L.; Wang, H.; Li, D.; Bai, Z.; Zhang, Y.; Sun, H.; Zhang, K.; Li, C. Exogenous melatonin accelerates seed germination in cotton (*Gossypium hirsutum* L.). *PLoS ONE* **2019**, *14*, e0216575. [CrossRef]
42. Bałabusta, M.; Szafrańska, K.; Posmyk, M.M. Exogenous melatonin improves antioxidant defense in cucumber seeds (*Cucumis sativus* L.) germinated under chilling stress. *Front. Plant Sci.* **2016**, *7*, 575.
43. Michel, B.E.; Kaufmann, M.R. The osmotic potential of polyethylene glycol 6000. *Plant Physiolol.* **1973**, *51*, 914–916. [CrossRef] [PubMed]
44. *ISTA International Rules for Seed Testing*; International Seed Testing Association: Zurich, Switzerland, 2020.
45. Mallone, J.P.; Muskett, A.E. *Seed Borne-Fungi. Description of 77 Fungus Species*; The International Seed Testing Association: Zurych, Switzerland, 1964.
46. Watanabe, T. *Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species*; CRC Press: Boca Raton, FL, USA, 2002.
47. Mathur, S.B.; Kongsdal, O. *Common Laboratory Seed Health Testing Methods for Detecting Fungi*; The International Seed Testing Association: Basserdorf, Switzerland, 2003.
48. Jalink, H.; van der Schoor, R. *SeedCalculator*, Version 2.1. License number: 100200122. Plant Research International: Wageningen, The Netherlands, 1999.

49. Hattori, A.; Migitaka, H.; Masayaki, I.; Itoh, M.; Yamamoto, K.; Ohtani-Kaneko, R.; Hara, M.; Suzuki, T.; Reiter, R.J. Identification of melatonin in plant seed its effects on plasma melatonin level sand binding to melatonin receptors in vertebrates. *Biochem. Mol. Biol. Int.* **1995**, *35*, 627–634. [PubMed]
50. Murch, S.J.; Erland, L.A.E. A systematic review of melatonin in plants: An example of evolution of literature. *Front. Plant Sci.* **2021**, *12*, 683047. [CrossRef] [PubMed]
51. Wei, L.X.; Lv, B.S.; Li, X.W.; Wang, M.M.; Ma, H.Y.; Yang, H.Y.; Yang, R.F.; Piao, Z.Z.; Wang, Z.H.; Lou, J.H.; et al. Priming of rice (*Oryza sativa* L.) seedlings with abscisic acid enhance seedling survival, plant growth, and grain yield in saline-alkaline paddy fields. *Field Crops Res.* **2017**, *203*, 86–93. [CrossRef]
52. Cao, Q.; Li, G.; Cui, Z.; Yang, F.; Jiang, X.; Diallo, L.; Kong, F. Seed priming with melatonin improves the seed germination of waxy maize under chilling stress via promoting the antioxidant system and starch metabolism. *Sci. Rep.* **2019**, *9*, 15044. [CrossRef]
53. Posmyk, M.M.; Kuran, H.; Marciniak, K.; Janas, K.M. Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. *J. Pineal Res.* **2008**, *45*, 24–31. [CrossRef]
54. Posmyk, M.; Bałabusta, M.; Wieczorek, M.; Śliwińska, E.; Janas, K.M. Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress. *J. Pineal Res.* **2009**, *46*, 214–223. [CrossRef]
55. Tiryaki, I.; Keles, H. Reversal of the inhibitory effect of light and high temperature on germination of *Phacelia tanacetifolia* seeds by melatonin. *J. Pineal Res.* **2012**, *52*, 332–339. [CrossRef]
56. Akbari, A.G.; Heshmati, S.; Soltani, E.; Dehaghi, M.A. Influence of seed priming on seed yield, oil content and fatty acid composition of safflower (*Carthamus tinctorius* L.) grown under water deficit. *Int. J. Plant Prod.* **2020**, *14*, 245–258. [CrossRef]
57. Jiang, X.W.; Li, H.Q.; Song, X.Y. Seed priming with melatonin effects on seed germination and seedling growth in maize under salinity stress. *Pak. J. Bot.* **2016**, *48*, 1345–1352.
58. Rosińska, A.; Dorna, H.; Szopińska, D. Effects of osmopriming on germination, vigour and health of *Silybum marianum* (L.) Gaertn. seeds. *Acta Sci. Pol. Hortorum Cultus* **2017**, *16*, 13–25. [CrossRef]
59. Rhaman, M.S.; Rauf, F.; Tania, S.S.; Khatun, S. Seed priming methods: Application in field crops and future perspectives. *Asian J. Res. Crop Sci.* **2020**, *5*, 8–19. [CrossRef]
60. Iranshahy, M.; Etemad, L.; Shakeri, A.; Badibostan, H.; Karimi, G. Protective activity of melatonin against mycotoxins-induced toxicity: A review. *Toxicol. Environ. Chem.* **2020**, *101*, 435–450. [CrossRef]
61. Hernández-Ruiz, J.; Giraldo-Acosta, M.; El Mihyaoui, A.; Cano, A.; Arnao, M.B. Melatonin as a possible natural anti-viral compound in plant biocontrol. *Plants* **2023**, *12*, 781. [CrossRef]
62. Janas, K.M.; Ciupińska, E.; Posmyk, M.M. Melatonin applied by hydropriming as a biostimulator improving sweet corn (*Zea mays* L.) seedling growth in abiotic stress condition. In *Progress in Environmental Science and Technology*; Li, S., Wang, Y., Ca, F., Huang, P., Zhang, Y., Eds.; Science Press: Princeton Junction, NJ, USA, 2009; Volume 2, pp. 383–388.
63. Posmyk, M.M.; Janas, K.M. Melatonin in plants. *Acta Physiol. Plant.* **2009**, *31*, 1–11. [CrossRef]
64. Altaf, M.A.; Shahid, R.; Ren, M.X.; Mora-Poblete, F.; Arnao, M.B.; Naz, S.; Anwar, M.; Altaf, M.M.; Shahid, S.; Shakoor, A.; et al. Phytomelatonin: An overview of the importance and mediating functions of melatonin against environmental stresses. *Physiol. Plant.* **2021**, *172*, 820–846. [CrossRef]
65. Arnao, M.B.; Hernández-Ruiz, J. Melatonin as a Chemical Substance or as Phytomelatonin Rich-Extracts for Use as Plant Protector and/or Biostimulant in Accordance with EC Legislation. *Agronomy* **2019**, *9*, 570. [CrossRef]
66. Chen, Q.; Qi, W.B.; Reiter, R.J.; Wei, W.; Wang, B.M. Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of *Brassica juncea*. *J. Plant Physiol.* **2009**, *166*, 324–328. [CrossRef]
67. Bai, Y.; Xiao, S.; Zhang, Z.; Zhang, Y.; Sun, H.; Zhang, K.; Wang, X.; Bai, Z.; Li, C.; Liu, L. Melatonin improves the germination rate of cotton seeds under drought stress by opening pores in the seed coat. *PeerJ* **2020**, *8*, e9450. [CrossRef]
68. Chipenete, G.H.N.; dos Santos Dias, D.C.F.; Pinheiro, D.T.; da Silva, L.J.; Pazzin, D.; da Silva, A.L. Carrot seeds vigor on plant performance and crop yield. *Rev. Verde* **2021**, *16*, 48–59. [CrossRef]
69. Guo, Y.; Li, D.; Liu, L.; Sun, H.; Zhu, L.; Zhang, K.; Zhao, H.; Zhang, Y.; Li, A.; Bai, Z.; et al. Seed priming with melatonin promotes seed germination and seedling growth of Triticale hexaploide L. under PEG-6000 induced drought stress. *Front. Plant Sci.* **2022**, *13*, 932912. [CrossRef] [PubMed]
70. Hanci, F. The effect of L-tryptophan and melatonin on seed germination of some cool season vegetable species under salinity stress. *Düzce Üniversitesi Bilim Ve Teknol. Derg.* **2019**, *7*, 1879–1891. [CrossRef]
71. Simlat, M.; Ptak, A.; Skrzypek, E.; Warchoł, M.; Morańska, E.; Piórkowska, E. Melatonin significantly influence seed germination and seedling growth of Stevia rebaudiana Bertoni. *PeerJ* **2018**, *6*, e5009. [CrossRef] [PubMed]
72. Arnao, M.B.; Hernández-Ruiz, J. The physiological function of melatonin in plants. *Plant Signal. Behav.* **2006**, *1*, 89–95. [CrossRef]
73. Yin, L.; Wang, P.; Li, M.; Ke, X.; Li, C.; Liang, D.; Wu, S.; Ma, X.; Li, C.; Zou, Y.; et al. Exogenous melatonin improves Malus resistance to Marssonina apple blotch. *J. Pineal Res.* **2013**, *54*, 426–434. [CrossRef]
74. Zhang, S.; Liu, S.; Zhang, J.; Reiter, R.J.; Wang, Y.; Qiu, D.; Luo, X.; Rehman Khalid, A.; Wang, H.; Feng, L.; et al. Synergistic anti-oomycete effect of melatonin with a biofungicide against oomycetic black shank disease. *J. Pineal Res.* **2018**, *65*, e12492. [CrossRef]
75. Lee, H.; Byeon, Y.; Back, K. Melatonin as a signal molecule triggering defense responses against pathogen attack in Arabidopsis and tobacco. *J. Pineal Res.* **2014**, *57*, 262–268. [CrossRef]

76. Liu, C.; Chen, L.; Zhao, R.; Li, R.; Zhang, S.; Yu, W.; Sheng, J.; Shen, L. Melatonin induces disease resistance to Botrytis cinerea in tomato fruit by activating jasmonic acid signaling pathway. *J. Agric. Food Chem.* **2019**, *67*, 6116–6124. [CrossRef]
77. Zhao, H.; Xu, L.; Su, T.; Jiang, Y.; Hu, L.; Ma, F. Melatonin regulates carbohydrate metabolism and defenses against Pseudomonas syringae pv. tomato DC3000 infection in Arabidopsis thaliana. *J. Pineal Res.* **2015**, *59*, 109–119. [CrossRef]