

## Article

# Evaluation of the Use of Oregano and Coconut Hydrolates to Improve Onion Seed Quality

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**Abstract:** Onion seeds are often contaminated by pathogenic fungi, such as *Botrytis* spp., *Fusarium* spp., which decrease seed quality. The usage of hydrolates is an alternative method to chemical treatment, and is safe for the natural environment, human health and life. The aim of the experiment was the determination of the effect of treatment with oregano and coconut hydrolates on the quality of onion seeds. Germination, vigour and seed health of two samples of onion seeds were tested. Seed germination was evaluated according to ISTA Rules, seed health by agar test and vigour by seed speed and uniformity of germination. Seeds were treated with hydrolate solutions at concentrations of 10, 20, 50 and 100%; untreated seeds and seeds soaking in water and treated with fungicide were control. Generally, the use of hydrolates improved the germination capacity at first and final count for both analyzed samples. After treating with hydrolate solutions, less abnormal diseased seedlings were also observed. Higher concentrations of hydrolates were effective in the limitation of the incidence of fungi *A. alternata*, *Cladosporium* spp. and *Fusarium* spp., either by complete elimination or a reduction of their presence on the seeds.

**Keywords:** hydrolates; hydrosols; *Allium cepa*; seed germination; seed vigour; seed health



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## 1. Introduction

The onion (*Allium cepa* L.) is a biennial bulb crop, belonging to the Amaryllidaceae family. It is one of the longest cultivated and used in food industry vegetable in the world, and the first report about onion appeared around 2600–2100 B.C., in Sumerian writings [1]. Onion seeds have poor storage potential and are strongly influenced by the seed vigour. As vigour is lost, germination capacity decreases and the seeds become more susceptible to fungal disease. Contaminations with storage fungi *Aspergillus* and *Penicillium*, which have been frequently recorded on onion seeds [2–5], have to be considered as additional factors causing fast deterioration of onion seed quality. Additionally, onion seeds can be a source of seed-borne pathogens *Botrytis allii* Munn, *B. byssoidea* Walker, *B. cinerea* Pers. and *Fusarium* spp., which are responsible for onion diseases such as onion neck rot, grey mould, seedling damping-off and Fusarium basal rot [6]. These fungi may cause a significant reduction in the quantity and quality of the crop. Nowadays, methods of effective seed treatment are currently being researched to replace the use of fungicides. These methods could significantly improve seed quality, reduce pathogen development and be harmless to the environment and human health. There have been reports on the use of essential oils for seed treatment, e.g., basil, fir, lemongrass, pine, sage and thyme on onion seed quality [7,8]. It has been shown that they can improve germination and seed vigour parameters and effectively inhibit pathogen development. Dorna et al. [7] reported that treating seeds with fir oil at the concentration of 0.2  $\mu\text{L cm}^{-3}$  for 6 h effectively controlled *Botrytis allii*, *B. cinerea* and *Fusarium* spp. and improved seed germination. Lozada et al. [8] observed that essential oils of basil, citronella, lemongrass, thyme and sage inhibited spore germination of *C. gleosporioides* f. sp. *cepae*. On the other hand, a high concentration of essential oils can completely inhibit seed germination [9]. Hydrolate (Hys) is the hydrophilic

fraction produced during essential oil (EO) steam distillation, and usually contains less than  $1 \text{ g L}^{-1}$  (i.e., 0.10%) of water-soluble aromatic compounds from the essential oil. The volatile components dissolved in hydrolates are mainly monoterpene alcohols, aldehydes, ketones and sesquiterpene alcohols. These plant-based preparations have antimicrobial, antifungal and antioxidant properties, are safe and do not need to be diluted before use. Therefore, hydrolates can be used for other applications such as environmental, entomological and agronomic applications [10,11].

In the experiment, two commercial hydrolates, oregano (*Origanum vulgare* L.) and coconut (*Cocos nucifera* L.), were used. These hydrolates were chosen due to their properties and lack of toxicity. The active substances present in oregano, carvacrol and thymol are responsible for its antioxidant, antibacterial and antifungal properties. Khan et al. [12] analysed by GC/MS the essential oil and aqueous distillate of *O. vulgare*. In both, they noted the presence of carvacrol and thymol. Carvacrol was the main active substance in both analysed products. Authors stated that carvacrol was responsible for antibacterial activity against the Gram-positive bacteria *Micrococcus luteus* and *Staphylococcus aureus*, and Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. In the case of coconut hydrolate, lauric acid and peptides AMP are responsible for antimicrobial activity [13,14]. Previous research confirmed that lauric acid was effectively inhibited the radial growth of *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. [15–17].

There is lack of data on how oregano and coconut hydrolates impact onion seed quality, especially seed health, which is crucial to vegetable production. Only the positive impact of coconut water on *Phalenopsis* and papaya seed germination were observed [18,19], but onion seeds were not treated before. The aim of our experiment was to evaluate the possibility of treatment with oregano and coconut hydrolates of onion seeds and the impact on the seed quality.

## 2. Materials and Methods

The standard seeds of two onions, (*Allium cepa* L.) cultivars Octavia (sample I) and Wolska TOR (sample II), obtained from a Polish seed company, were used in the experiment. Seed samples varied in quality, especially germination capacity and settlement by pathogenic fungi *Botrytis cinerea* and *B. allii*.

Coconut and oregano hydrolates, produced by Ajeden Sp. z o. o., were dissolved in distilled water to obtain 10, 20 and 50% solutions. Seeds were soaked in aqueous solutions of coconut and oregano hydrolates and undiluted hydrolates for 30 min. After soaking, seed surfaces were dried on a sterile blotter for 5 min. Subsequently, seeds were put to dry in plastic, semi-open Petri dishes for 24 h and 45% RH. Untreated seeds, seeds treated with fungicide Dithane Neo Tec 75WG (a.s. mancozeb) at dose of  $5 \text{ g} \cdot \text{kg}^{-1}$  of seeds and seeds soaked in water were controls.

### 2.1. Germination Test

A Seed germination test was conducted at  $20^\circ \text{C}$  in darkness, on six replicates of 50 seeds from each treatment. Seeds were placed in Petri dishes on six layers of blotter paper moistened with distilled water. After 6 days of incubation, seed germination at the first count (only the percentage of normal seedlings) was determined, whereas after 12 days, germination at the final count (percentage of normal seedlings) was evaluated. Badly decayed seedlings were removed in order to reduce the risk of secondary infection from the test at first count. Moreover, after 12 days of incubation, the percentage of diseased and deformed seedlings and the percentages of fresh and dead seeds were determined. Additionally, during a vigour test, the percentage of germinated seeds (Gmax) was also evaluated [20].

### 2.2. Vigour Test

A seed vigour test was conducted at  $20^\circ \text{C}$  in darkness, on 6 replicates of 50 seeds from each treatment. Seeds were placed in Petri dishes on six layers of blotter paper moistened

with distilled water. Germinated seeds were counted every day, until no new germs occurred, and were removed from Petri dishes. The seeds were considered as germinating when the radicle was at least 1 mm long. The following parameters were calculated:  $T_{10}$ ,  $T_{50}$  (time required to germinate 1 and 50% seeds of  $G_{max}$ ) and  $U_{75-25}$  (time required to germinate from 25 to 75% seeds of  $G_{max}$ ).

### 2.3. Seed Health Test

Two hundred seeds from each treatment, and 4 replicates of 50 seeds, were analysed by a potato-dextrose-agar (PDA) test. The seeds were surface disinfected with 1% of aqueous solution of sodium hypochlorite ( $NaClO$ ) for 5 min, and rinsed with sterile water and surface dried on sterile blotter paper. Then, seeds were placed in Petri dishes on PDA with an addition of 100 ppm streptomycin sulphate to prevent growth of bacteria, at 10 seeds per a dish, and then incubated at 20 °C under alternating cycle of 12 h NUV light and 12 h darkness for 10 days. Seeds were observed under a stereo-microscope (magnification 50×) and a compound microscope if necessary. Fungi on the seeds were identified based on the performance of the colonies, fungus sporulation characteristics and colour [21–23]. The percentages of seeds infested with individual fungi and seeds free of fungi were calculated.

All results were analysed using STAT software, by means of one-way analysis of variance after transforming percentage values according to Bliss' formula:  $y = \arcsin [\sqrt{x/100}]$ . Means were compared with the Duncan's multiple range test. Parameters characterising seed vigour speed of germination ( $T_{10}$  and  $T_{50}$ ) and uniformity of germination  $U_{75-25}$  were evaluated using the SeedCalculator 2. 1. [24].

## 3. Results

### 3.1. Germination Test

Generally, there was no effect of seed treatment with hydrolates on the  $G_{max}$  parameter. Only after treating the seeds of sample I (cv. Octavia) with a 20% solution of oregano hydrolate, a significantly higher percentage of germinated seeds was found ( $G_{max}$ ) in comparison with untreated seeds. However, the effect of using hydrolates on germination parameters was significant. The germination capacity at first and final counts were significantly improved in comparison with untreated seeds. These differences are particularly evident for germination capacity at first count, which was 51.7% for sample I and 40.3% for sample II (cv. Wolska TOR). The use of hydrolate solutions increased this parameter by 12–35% for sample I and by 18.4–33% for the second one. In the case of sample I, it was found that solutions of coconut hydrolate at concentrations of 20 and 100%, and oregano, regardless of the concentration, improved germination at the fungicide level (Tables 1 and 2).

The presence of fungi in the seeds was the cause of poor seed germination. A high percentage of abnormal seedlings with disease symptoms was recorded in both samples, 18.7% for sample I and 22.7% for sample II. Additionally, sample II was characterized by 13.0% percentage dead seeds. After seed treatment with hydrolates, except the use of coconut hydrolate at a concentration of 10% for sample I, a significant reduction of the percentage of abnormal diseased seedlings was observed. Hydrolates were as effective as the fungicide. Generally, the impact on the percentage of dead seeds was not observed. Only in the case of sample II, soaking seeds in the oregano hydrolate at concentrations of 10 and 50% significantly reduced the number of dead seeds in comparison with untreated seeds. They were observed at the fungicide level (Tables 1 and 2).

The coconut hydrolate did not influence the percentage of abnormal deformed seedlings. In the case of oregano hydrolate, it was found that solutions at concentrations of 10 and 20% effectively reduced the value of this parameter compared with untreated seeds treated with fungicide and soaked in distilled water. The percentage of fresh seeds of sample I was 1.0% and none of the treatments had an effect on it. The value of this parameter for untreated seeds of sample II was 0.7%, and by soaking the seeds in only 100% coconut hydrolate significantly increased the percentage of fresh seeds (4.0%) (Tables 1 and 2).

**Table 1.** Effects of coconut and oregano hydrolates treatment on the seed germination of onion seeds of sample I (%).

Seed Treatment	Gmax **		Germination Capacity				Abnormal Diseased Seedlings		Abnormal Deformed Seedlings		Fresh Seeds		Dead Seeds	
			At First Count		At Final Count									
U *	91.7	b	51.7	e	67.7	d	18.7	a	6.7	a	1.0	ab	6.0	ab
F	96.7	ab	82.0	ab	86.7	a	4.3	d-f	4.0	ab	0.7	ab	4.3	bc
W	97.0	ab	60.0	de	75.3	b–d	16.7	a	5.3	ab	0	b	2.7	c
C10	96.3	ab	67.7	cd	77.7	bc	12.7	ab	4.3	ab	0.3	ab	4.3	bc
C20	94.3	b	72.7	bc	82.0	a–c	7.3	b–d	6.3	ab	0	b	4.3	bc
C50	94.3	b	63.7	cd	73.3	cd	8.0	bc	5.7	ab	2.0	a	11.0	a
C100	96.3	ab	78.7	ab	83.0	bc	4.7	c–f	4.0	ab	2.3	a	5.7	ab
O10	97.3	ab	86.3	a	89.0	a	3.7	f	1.0	c	1.0	ab	4.3	bc
O20	98.7	a	83.7	a	87.7	a	3.7	f	1.0	c	1.7	a	6.0	ab
O50	96.0	ab	77.3	a-c	87.0	a	6.7	b–e	3.7	a-c	0	b	3.0	bc
O100	96.3	ab	80.7	ab	87.3	a	4.7	c–f	2.3	bc	0	b	5.7	ab

\* U—untreated seeds (control), F—fungicide, W—water control, C<sub>10</sub>—seeds soaked in 10% coconut hydrolate solution for 30 min, C<sub>20</sub>—seeds soaked in 20% coconut hydrolate solution for 30 min, C<sub>50</sub>—seeds soaked in 50% coconut hydrolate solution for 30 min, C<sub>100</sub>—seeds soaked in 100% coconut hydrolate for 30 min, O<sub>10</sub>—seeds soaked in 10% oregano hydrolate solution for 30 min, O<sub>20</sub>—seeds soaked in 20% oregano hydrolate solution for 30 min, O<sub>50</sub>—seeds soaked in 50% oregano hydrolate solution for 30 min, O<sub>100</sub>—seeds soaked in 100% oregano hydrolate for 30 min, \*\* Gmax—the percentage of germinating seeds. Means in the columns followed by the same letter are not significantly different at  $\alpha = 0.05$  level.

**Table 2.** Effects of coconut and oregano hydrolates treatment on the seed germination of onion seeds of sample II (%).

Seed Treatment	Gmax **		Germination Capacity				Abnormal Diseased Seedlings		Abnormal Deformed Seedlings		Fresh Seeds		Dead Seeds	
			At First Count		At Final Count									
U *	92.0	ab	40.3	c	56.3	c	22.7	a	4.3	a-c	0.7	bc	13.0	ab
F	94.3	a	60.0	b	69.7	ab	14.3	b	5.3	ab	1.7	b	9.0	a-c
W	86.3	b	58.3	b	68.7	b	14.3	b	5.3	ab	0	c	11.7	a-c
C10	89.3	b	63.7	ab	71.7	ab	14.0	b	2.3	a-c	0	c	11.7	a-c
C20	89.7	b	63.7	b	72.7	ab	13.0	b	4.0	a-c	0	c	10.0	a-c
C50	88.0	b	61.7	b	71.7	ab	11.0	b	2.7	a-c	0	c	14.7	a
C100	89.0	b	58.7	ab	73.3	ab	12.7	b	2.0	bc	4.0	a	8.0	bc
O10	90.7	b	68.3	ab	79.3	a	10.7	b	1.0	c	2.0	b	7.0	c
O20	86.0	b	73.3	a	78.0	ab	8.3	b	2.0	bc	2.0	b	8.0	bc
O50	89.7	b	64.3	ab	76.0	ab	10.3	b	6.7	a	0	c	7.0	c
O100	89.3	b	63.0	ab	76.7	ab	9.3	b	5.7	ab	0	c	8.3	bc

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

### 3.2. Vigour Test

Usage of coconut and oregano hydrolates at a concentration of 10% accelerated the seed germination of sample I compared with the fungicide treatment. It was also found that at this concentration, coconut hydrolate reduced the value of the T<sub>50</sub> parameter and oregano hydrolate and the values of T<sub>10</sub> and T<sub>50</sub> parameters in comparison with untreated and soaked in water seeds. Seeds soaked in 100% coconut hydrolate were germinated in the most uniform way, and the time required for germination from 25 to 75% of the Gmax was shortened for untreated, treated with fungicide and soaked in water seeds at about, 0.3, 0.5 and 0.3 days, respectively. Seeds soaked in coconut hydrolate at concentrations of 20 and 50% and oregano at 20% germinated more uniformly than seeds treated with the fungicide (Table 3).

**Table 3.** Effects of coconut and oregano hydrolates treatment on the speed and uniformity of seed germination of onion seeds of sample I (days).

Seed Treatment	T <sub>10</sub> **		T <sub>50</sub>		U <sub>75-25</sub>	
U *	1.5	a–c	2.3	b–d	1.0	ab
F	1.6	ab	2.7	a	1.2	a
W	1.6	a–c	2.4	bc	1.0	ab
C10	1.3	cd	1.9	f	1.1	ab
C20	1.7	a	2.3	cd	0.9	bc
C50	1.5	a–c	2.1	d–f	0.9	bc
C100	1.7	ab	2.1	d–f	0.7	c
O10	1.2	d	2.0	ef	1.1	ab
O20	1.5	a–c	2.2	c–f	0.8	bc
O50	1.7	a	2.6	cd	1.1	ab
O100	1.4	b–d	2.2	c–e	1.0	ab

\* for explanations see Table 1. \*\* T<sub>10</sub>—time to 10% of G<sub>max</sub> (the percentage of germinating seeds), T<sub>50</sub>—time to 50% of G<sub>max</sub>, U<sub>75-25</sub>—time between 25 and 75% of G<sub>max</sub>. Means in columns followed by the same letter are not significantly different at  $\alpha = 0.05$  level.

In the case of sample II, acceleration of the germination was observed when seeds were treated with hydrolates solutions at lower concentrations. The lowest value of the T<sub>10</sub> was noted after soaking seeds in a 20% oregano hydrolate solution. This parameter was lower than for untreated, treated with fungicide and soaked in distilled water at about 1.3, 1.3 and 1.4 days, respectively. Seeds treated with 10% coconut hydrolate and oregano hydrolate with concentrations of 10 and 20% germinated faster, and the values of the T<sub>50</sub> were significantly lower than all tested controls. Compared to the untreated seeds, the seeds treated with hydrolates did not germinate more uniformly. However, it was found that after soaking in 20% coconut hydrolate and in 10, 20 and 50% oregano hydrolate solutions, the seeds germinated more uniformly than the seeds treated with the fungicide (Table 4).

**Table 4.** Effects of coconut and oregano hydrolates treatment on the speed and uniformity of seed germination of onion seeds of sample II (days).

Seed Treatment	T <sub>10</sub> **		T <sub>50</sub>		U <sub>75-25</sub>	
U *	1.9	b	2.7	a–c	0.9	a–d
F	1.9	b	2.9	a	1.2	a
W	2.0	b	2.7	bc	0.9	a–d
C10	1.9	b	2.5	de	1.0	a–c
C20	2.0	b	2.7	b–d	0.8	cd
C50	1.8	b	2.6	c–e	1.1	ab
C100	1.9	b	2.7	bc	0.9	a–d
O10	1.8	b	2.5	e	0.9	b–d
O20	0.6	c	2.5	e	0.8	d
O50	2.1	a–c	2.7	ab	0.9	b–d
O100	1.9	b	2.7	a–c	1.1	ab

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

### 3.3. Seed Health Test

The untreated seeds of sample I were infested with *Alternaria alternata* (21.0%), *Botrytis allii* (24.0%), *B. cinerea* (26.5%), *Cladosporium* spp. (61.5%), *Fusarium* spp. (39.5%) and *Stemphylium botryosum* (6.0%). The oregano hydrolate solutions at concentrations of 50 and 100% effectively reduced the incidence of the following fungi: *A. alternata*, *Botrytis allii*, *B. cinerea* and fungi from genera *Cladosporium* and *Fusarium*. These solutions were as effective as fungicide or even better. Additionally, the highest percentage of seeds free of fungi was noted when seeds were soaked in 50% oregano hydrolate solution (24.5%) and 100% oregano hydrolate (28.0%). Seeds free of fungi were also recorded after soaking



the seeds in 50 and 100% solutions of coconut hydrolate, at 11.0 and 20.0%, respectively. Untreated seeds and seeds treated with the fungicide seeds free of fungi were not observed. Almost all hydrolate solutions used in the experiment reduced the incidence of *B. cinerea* and *Cladosporium* spp. It was found that seed settlement by *Cladosporium* spp. decreased with increasing concentrations of the hydrolate solutions. A reduction in the number of seeds infected by fungi from the genus *Fusarium* was observed after seed treatment with a fungicide and coconut hydrolate at concentrations of 20, 50 and 100%. After usage of 20% oregano hydrolate solution, a significantly higher percentage of seeds occupied by *Fusarium* spp. was observed. Most of the applied methods, also treating by fungicide with fungicide, significantly increased the occurrence of *Stemphylium botryosum*. No increase in seed colonisation by this fungus was found, but was found only after soaking the seeds in water and 100% oregano hydrolate (Table 5).

**Table 5.** Effects coconut and oregano hydrolates treatment on the incidence of fungi on onion seeds of sample I and seeds free of fungi (%).

Seed Treatment	<i>Alternaria alternata</i>		<i>Botrytis allii</i>		<i>Botrytis cinerea</i>		<i>Cladosporium</i> spp.		<i>Fusarium</i> spp.		<i>Stemphylium botryosum</i>		Seeds Free of Fungi	
U *	21.0	a	24.0	b	26.5	a	61.5	a	39.5	b	6.0	a	0	c
F	5.5	cd	23.5	b	3.5	b–d	11.5	b	15.5	d	34.5	a–c	0	c
W	13.5	a–c	29.5	ab	14.0	a–c	61.0	a	42.5	ab	8.5	ef	0.5	c
C10	14.5	a–c	31.5	ab	1.5	cd	35.5	b	42.5	ab	38.5	ab	1.5	c
C20	14.0	a–c	31.0	ab	12.0	ab	26.0	b	25.5	c	44.5	a	2.0	c
C50	9.5	b–d	27.5	ab	7.5	b–d	16.0	c	14.0	d	36.5	ab	11.0	b
C100	6.5	d	23.5	b	5.0	b–d	15.5	c	6.5	e	34.5	ab	20.0	ab
O10	15.0	ab	36.5	a	2.5	b–d	48.5	a	36.0	b	24.0	b–d	1.0	c
O20	10.0	b–d	26.5	ab	9.5	bc	49.0	a	52.5	a	17.5	de	0	c
O50	2.0	e	8.0	c	0	d	10.0	c	9.0	de	19.0	cd	24.5	ab
O100	0.5	e	1.0	d	0	d	1.5	d	4.5	e	7.5	ef	28.0	a

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

The untreated seeds of sample II were occupied by the greatest extend by *Alternaria alternata* (22.5%), *Cladosporium* spp. (21.0%) and *Fusarium* spp. (42.5%). All applied methods effectively reduced the incidence of *A. alternata*, and the best were soaking seeds in 50 and 100% oregano hydrolate. Seeds treated with solutions of coconut hydrolate at concentrations of 10 and 20% and all solutions of oregano hydrolate were significantly less occupied by *Botrytis allii* compared with untreated seeds and seeds treated with fungicide. Complete elimination of *Botrytis cinerea* from seeds was observed after treatment with 100% coconut hydrolate, 50% oregano hydrolate and fungicide. All of the applied treatments, except oregano hydrolate at concentration 10%, significantly lowered the percentage of incidence of *Cladosporium* spp. compared with untreated seeds and water control. Treating seeds with fungicide and 100% oregano hydrolate reduced to the greatest extent the incidence of *Fusarium* spp., by about 26 and 37%, respectively, whereas soaking in distilled water increased it by about 57%. As the concentration of the hydrolates solutions increased, a lower percentage of infested seeds was observed. However, application of coconut hydrolate solutions at concentration 10 and oregano hydrolate at concentrations of 10 and 20% increased seed infection by this fungus compared to the untreated seeds. Seed soaked in water resulted in the lowest level of incidence of *Stemphylium botryosum* respective to control and fungicide. Meanwhile, after soaking the seeds in 20 and 100% coconut hydrolate solutions, a significantly higher percentage of seeds occupied by that fungus was observed. Only the application of 100% oregano hydrolate and fungicide significantly increased the number of seeds free of fungi (Table 6).

**Table 6.** Effects coconut and oregano hydrolates treatment on the incidence of fungi on onion seeds of sample II and seeds free of fungi (%).

Seed Treatment	<i>Alternaria alternata</i>		<i>Botrytis allii</i>		<i>Botrytis cinerea</i>		<i>Cladosporium spp.</i>		<i>Fusarium spp.</i>		<i>Stemphylium botryosum</i>		Seeds Free of Fungi	
U *	22.5	a	6.0	ab	12.5	a	21.0	a	42.5	c	11.5	cd	0	c
F	8.0	b	10.5	a	0	d	0	f	16.5	e	23.0	bc	9.0	ab
W	14.5	ab	0	d	2.0	b–d	21.0	a	99.5	a	3.0	e	0	c
C10	13.5	ab	1.5	cd	6.0	ab	10.0	bc	81.5	b	7.5	de	0.5	c
C20	9.5	b	0.5	d	3.0	b–d	0	f	48.5	c	36.0	a	0	c
C50	7.0	b	6.0	ab	5.0	a–c	5.0	cd	47.0	c	21.5	bc	1.0	c
C100	9.0	b	3.0	bc	0	d	1.0	ef	39.5	cd	33.5	ab	4.0	bc
O10	10.0	b	1.5	cd	5.0	a–c	14.0	ab	76.5	b	12.5	cd	0	c
O20	7.0	b	1.5	cd	0.5	cd	9.0	bc	70.5	b	16.0	cd	0	c
O50	0.5	c	0.5	d	0	d	2.0	de	26.0	d	9.5	de	3.0	bc
O100	1.0	c	1.5	cd	8.0	a–c	0.5	ef	5.5	e	18.5	c	16.5	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

The main aim of this study was to check how the treatment of plant origin preparations (coconut and oregano hydrolates) influences the quality of onion seeds. Chemical plant protection products are being systematically withdrawn, so new solutions are being researched. Plant origin preparations, as essential oils and hydrolates, are environmentally safe and harmless to human and animal health and life.

Seeds of both samples treated with coconut hydrolate were characterized by higher germination capacity at first and final counts than untreated ones. In the literature, the beneficial effects of coconut water on the seed germination are reported. According to Shekarriz et al. [23], coconut water improved the seed germination of *Phalaenopsis* orchids. Zainudin and Adini [24] reported that after immersion of papaya seeds in coconut water at concentrations 60 and 80% for 8 h, a higher percentage of germination, growth rate and vigour index were observed. The improvement of these parameters as well as the possibility of breaking seed dormancy are related to the content of the phytohormones—auxins, gibberellins, cytokinins, pyridoxine, nicotinic acid and thiamine in coconut water [25] and nutritional elements, potassium and calcium. Potassium and calcium stimulate cell division and elongation and the growth of the plant [26]. Phytohormones, as cytokinins, are also responsible for growth promoting, divide cells in the roots and growing shoots [27]. Soaking in coconut water activated cell metabolism and affected the growth of seedlings [24].

Demirbaş and Karaca [28] used the *Origanum onites* hydrolate to control *Phytophthora* spp. in the soil. They found that solutions containing oregano water over a 10% rate significantly decreased pathogen populations and positively influenced seed germination and seedlings emergence. Atak et al. [29] evaluated an effect of oregano oil on germination and seedling growth of durum wheat. After treating seeds with extracts, the author observed a significant increase in germination percentage, germination index, seedling shoot length, seedling root length and seedling fresh weight, apart from that delay on mean germination time upon increased oil dose. In the present study, germination at first and final counts of seeds treated with oregano hydrolate solutions were better than untreated seeds, especially in the case of seeds of sample I. The phytotoxic effect of a high concentration of hydrolate solutions was not observed. In the case of hydrolates, it is possible to use high concentrations because the active substances, such as thymol or carvacrol, which are toxic to seedlings, are dissolved in significantly lower concentrations. However, the volatiles contained in the hydrolates are more effective in inhibiting the microbial growth, because they are active at the lower concentrations [30]. When using hydrolates to improve the quality of seeds, it is important to be aware of their chemical composition. Politi et al. [31] reported that Lavandin flowers hydrolate, which contains a high content of linalool and 4-terpineol, and shows a strong inhibition effect on radish seeds germination.

In this study, it was shown that the presence of fungi from the genus *Fusarium*, after treating seeds with higher concentrations of hydrolates, in both samples was smaller. Kocic-Tanackov et al. [32] observed inhibition of the growth of *Fusarium* and *Penicillium*, isolated from cake and ready for use in salads of different vegetables. The authors added the oregano extract at concentrations of 0.35, 0.7, 1.5 and 2.5 mL/100 mL to PDA medium. The inhibition of colony growth was higher as the oregano extract concentration was increased. They also observed changes in fungal macro- and micro-morphology. The compounds in oregano are responsible for its antifungal properties, the greatest important being carvacrol and thymol [33,34]. *p*-Cymene and  $\gamma$ -terpinene, which are bioprecursors of carvacrol and thymol, were evaluated as weaker antimicrobial agents [35,36]. Chemical compounds in hydrolates, especially phenolic, disrupt fungal membranes and cell walls and action of their enzymes [37]. Previous studies demonstrated that carvacrol showed antifungal activity against following fungi: *Aspergillus niger*, *A. flavus*, *Alternaria alternata*, *Penicillium rubrum*, *Trichoderma viride* and *Candida* sp. [38]. Lima et al. [39] suggested that carvacrol is able to bind to sterols in the fungal membrane, leading to disruption of the cell membrane structure and death of the fungus. The inhibitory effect of oregano hydrolate on the mycelial growth of *Aspergillus parasiticus* was observed by Özcan [40]. In addition, the use of oregano hydrolate can reduce the production of mycotoxins by fungi, as fumonisin B1 produced by *Fusarium* spp. [41]. Salmeron et al. [42] found that the addition of ground oregano and thyme into the growth medium reduced the production of aflatoxins by *A. parasiticus*.

Perez-Gonzalez et al. [43] isolated from red tomatoes the *Alternaria alternata* and treated them with oregano oil (1, 5 and 10%) in in vitro conditions, which resulted in increased inhibition of up to 100% along with an increase in oregano oil concentration. In the present experiment, seeds of both samples, which were treated with oregano hydrolate, were characterized by a lower incidence of *A. alternata* with an increase in hydrolate concentration.

In the present experiment, after soaking seeds in the highest concentrations (50 and 100%) of coconut hydrolate, fewer fungi of the genus *Cladosporium* was also observed. For the coconut's antimicrobial activity, lauric acid [13] and antimicrobial peptides AMP, designated CnAMP1, CnAMP2 and CnAMP3 [14] are responsible. Plocková et al. [15] and Řiháková et al. [16,17] tested the lauric acid derivatives against *Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp., they observed two different types of antifungal effects. The first type involved the inhibition of spore germination and the second one was the inhibition of radial growth. On the other hand, Rukmini et al. [44] did not confirm antimicrobial activity of coconut water against *Streptococcus mutans*.

In the present study, generally antifungal activity was higher as higher concentrations of hydrolates were used. In the case of *Fusarium* spp., more seeds treated with lower doses of coconut and oregano hydrolates were occupied by these fungi than untreated ones. Similar observations were made by Boyraz and Özcan [45], whereby cumin, sater and pickling herb hydrolates at higher doses decreased mycelial growth by a greater extent or completely inhibited growth. On the other hand, the authors noted that rosemary and basil hydrolates at certain doses stimulated mycelial growth in comparison with control. Due to the different reaction of individual fungi to hydrolates and their concentrations, further research is advisable.

Current studies have shown a magnificent impact on seed health in both samples, especially with higher concentrations of coconut and oregano hydrolates. Due to their beneficial effects on germination and seed health, hydrolates can be recommended for seed treatment.

## 5. Conclusions

Generally, the use of hydrolates improved the germination capacity at first and final counts of onion seeds for both analyzed samples. After treating with hydrolates, fewer abnormal diseased seedlings were observed.



A reduction in the time, which is required for the germination of 10 and 50% of seeds of the total number of germinated seeds (Gmax), was observed after treatment seeds with a 10 and 20% solution of oregano hydrolate, respectively, for samples I and II.

Based on the results, it was proven that higher concentrations of both hydrolates were effective in the limitation of the incidence of fungi as *A. alternata*, *Cladosporium* spp. and *Fusarium* spp., either by complete elimination or reduction of their presence on the seeds.

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