

EFFECT OF TREATING LETTUCE (*Lactuca sativa* L.) SEEDS WITH SOLUTIONS OF ETHEREAL OILS FROM CAMPHOR TREE (*Cinnamomum camphora* L.) AND PATCHOULI PLANT (*Pogostemon cablin* Benth.) ON THEIR GERMINATION

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Abstract. One of the most important problems in horticultural seed science and technology nowadays is looking for new methods to improve seed quality. Especially interesting are the ones, in which compounds of natural origin are used. One of such groups is ethereal oils from plants. The commercial seed samples of lettuce (*Lactuca sativa* L. 'Dumka') were soaked in 9 different treatments of 5% alcoholic solution of camphor tree (*Cinnamomum camphora* L.) ethereal oil and 9 different treatments of 5% alcoholic solution of patchouli plant (*Pogostemon cablin* Benth.) ethereal oil. Then the seeds were routinely tested for seed germination, the length of the received seedlings was also measured and the seeds health status was evaluated. The tested ethereal oils had no effect on the germination of lettuce seeds. However, soaking the seeds in the ethereal oils solutions did decrease the percentage of dead seeds in the tested samples. Soaking of the seeds in the ethereal oils solutions also increased the percentage of healthy ungerminated seeds. Treating the seeds with the concentrations of the ethereal oils solutions, although had no effect on their germination, still shortened the length of seedlings. Moreover, the used ethereal oils lowered the amount of identified *Alternaria alternata* and *Cladosporium sphaerospermum* fungi on the seeds.

Key words: soaking of the seed, seed quality germination capacity, seed infestation and incidence of fungi

INTRODUCTION

Seeds, as agriculture materials, are of great importance. Good quality seed is one with inherited traits for good growth performance, shape, product quality, good germination ability and good storability as well as without pest and diseases [Lars 2004].

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Seed quality of most species is improved by preconditioning, cleaning, disinfection and through control of storage condition. In recent years, an increasing range of chemical seed treatments have also become available. It has long been known that steeping with or without one or more wetting treatments can improve seed germination performance [Halmer 2000]. In organic agriculture, people have developed a combination therapy by bringing together physical treatments, treatments with compounds of natural origin and microbial buffering of disinfected seed with antagonists. Plant ethereal oils, as the natural plant resources, have become one of the organic seed treatment methods in recent years.

The camphor tree (*Cinnamomum camphora* L.) is a lofty, evergreen tree, growing to a height of 50 or 60 feet, with a trunk of a proportionate diameter. Early in 1991, Mishra *et al.* discovered during screening of ethereal oils, the oil of *Cinnamomum camphora* was found to possess mycostatin effect against *Aspergillus flavus* at 4000 ppm. From the beginning of 21st century, an increasing number of studies regarding *Cinnamomum camphora* ethereal oil were done. Deng *et al.* [2004] showed that *Cinnamomum camphora* ethereal oil performed the best effect on fumigating maize weevil (*Sitophilus zeamais* L.). Wang [2007] illustrated that cinnamon ethereal oil presented better inhibition to the growth of *Botrytis cinerea*, *Alternaria solani*, *Cladosporium fulvum* and *Pseudoperonospora cubensis* as well as bacteria spore germination. Moreover, the applied *Cinnamomum camphora* oil was significantly toxic to seed germination of wheat at 500 µg/ml [Liu *et al.* 2006].

Patchouli (*Pogostemon cablin* Benth.) is an erect much-branched aromatic herb, up to 1.2 m high, with ovate crenate-serrate tomentose leaves, white flowers with purple streaks in whorls on terminal and axillary spikes [Daniel 2006]. It was found that the anti-infective properties of patchouli plant ethereal oil are particularly useful for fungal infection of the skin; it may also be of help for haemorrhoids [Tiran 2000]. Mo *et al.* [2004] found that when the concentrations of patchouli ethereal oil in the medium reached from 0.03% to 0.27%, 13 different kinds of plant pathogenic fungi were inhibited in various degrees. Thereinto, *Alternaria solani* and *Sclerotinia sclerotiorum* were completely inhibited when the patchouli ethereal oil was applied at 0.1%. Zeng *et al.* [2006] proved that patchouli ethereal oil possessed a relatively good prevention to the larva of *Pieris rapae* and *Plutella xylostella*. The goal of this research was to investigate if the applied plant ethereal oils can improve quality of lettuce seeds.

MATERIAL AND METHODS

Lettuce seeds (*Lactuca sativa* L., cv. 'Dumka') were provided by the Polish seed company "W. Legutko", seedlot No.: P. 714/32/220/LE798A. The seeds were stored in a fridge at 4 °C. Moisture content (MC) of them was 5.5%.

95% edible alcohol was bought from the Polish manufacturer 'Polmos'. It was diluted by distilled water. One concentration was applied in all seed soaking treatments:

– 5% alcoholic solution for 30 minutes – 5A30

Camphor tree ethereal oil was bought from the company "Aroma Show", located in the Hunan Province, China. The oil was 100% pure essential one. It was extracted from

wood through distillation in the *Sichuan* Province, China. Nine different camphor tree ethereal oil solutions were used in the experiment, the seeds were soaked in:

- 5% alcoholic solution of 0.1% camphor tree ethereal oil for 30 minutes – 0.1C30
- 5% alcoholic solution of 0.1% camphor tree ethereal oil for 30 minutes, then rinsed with sterilized water 3 times – 0.1C30R
- 5% alcoholic solution of 0.1% camphor tree ethereal oil for 20 minutes, then soaked in distilled water for 10 minutes – 0.1C20D10
- 5% alcoholic solution of 0.2% camphor tree ethereal oil for 30 minutes – 0.2C30
- 5% alcoholic solution of 0.2% camphor tree ethereal oil for 30 minutes, then rinsed with sterilized water 3 times – 0.2C30R
- 5% alcoholic solution of 0.2% camphor tree ethereal oil for 20 minutes, then soaked in distilled water for 10 minutes – 0.2C20D10
- 5% alcoholic solution of 0.4% camphor tree ethereal oil for 30 minutes – 0.4C30
- 5% alcoholic solution of 0.4% camphor tree ethereal oil for 30 minutes, then rinsed with sterilized water 3 times – 0.4C30R
- 5% alcoholic solution of 0.4% camphor tree ethereal oil for 20 minutes, then soaked in distilled water for 10 minutes – 0.4C20D10

Patchouli plant oil was bought from the company “Herb Shop” in Canton, China. The oil was 100% pure essential one. It was extracted from leaves through distillation in China. Nine different patchouli solutions were used in the experiment, the seeds were soaked in:

- 5% alcoholic solution of 0.005% patchouli plant ethereal oil for 30 minutes – 0.005P30
- 5% alcoholic solution of 0.005% patchouli plant ethereal oil for 30 minutes, then rinsed with sterilized water 3 times – 0.005P30R
- 5% alcoholic solution of 0.005% patchouli plant ethereal oil for 20 minutes, then soaked in distilled water for 10 minutes – 0.005P20D10
- 5% alcoholic solution of 0.01% patchouli plant ethereal oil for 30 minutes – 0.01P30
- 5% alcoholic solution of 0.01% patchouli plant ethereal oil for 30 minutes, then rinsed with sterilized water 3 times – 0.01P30R
- 5% alcoholic solution of 0.01% patchouli plant ethereal oil for 20 minutes, then soaked in distilled water for 10 minutes – 0.01P20D10
- 5% alcoholic solution of 0.02% patchouli plant ethereal oil for 30 minutes – 0.02P30
- 5% alcoholic solution of 0.02% patchouli plant ethereal oil for 30 minutes, then rinsed with sterilized water 3 times – 0.02P30R
- 5% alcoholic solution of 0.02% patchouli plant ethereal oil for 20 minutes, then soaked in distilled water for 10 minutes – 0.02P20D10

The lettuce seed MC was checked after putting them in the oven and dried for 1 hour at 130°C till it was constant.

For all the seeds, their germination was evaluated based on the routine International Seed Testing Association (ISTA) rules. Six replications of 50 seeds for each treatment were placed in Petri dishes containing 6 layers of moistened blotters with distilled water. After that, seeds germinated in a germination chamber at 20°C. Energy and capacity

of germination were recorded on the 4th and 7th day, respectively. The numbers of normal, abnormal seedlings, dead and healthy ungerminated seeds were counted. Germination energy was a measure of the rapidity of germination expressed as the percentage of seeds germinating within 4 days after setting in comparison with the number of seeds tested. Accordingly, it reflected germination rate, uniformity, vigour and viability [Bewley et al. 2006].

The growth of seedlings was characterized by the length of stem and root. According to Ren and He [2008], length of stem and root of seedlings were measured on the 4th day after planting tested seeds.

Blotter test was applied to detect the seedborne fungi and the presence of mycelium and fruiting bodies on the seed. Ten replicates of 20 seeds for each treatment were put in glass dishes on moist absorbent paper blotters of 6 layers. On the 1st day, they were incubated in darkness at the 20°C chamber for 24 hours. Subsequently, they were moved to a fridge at -20°C for next 24 hours to inhibit seed germination. On the 3rd day, they were kept in the 20°C chamber again under near ultra violet (NUV) light. On the 10th day, the detection of fruiting bodies by microscopic examination was done.

For statistical analyses, all received data was analyzed by means of the STAT programme followed by the Duncan's range test at $\alpha = 0.05$. Before the STAT, percentages were transformed according to $y = \arcsin[\sqrt{x/100}]$.

RESULTS

Seed germination after camphor tree ethereal oil solutions treatments. The carried out experiment proved that the use of this ethereal oil solutions did not affect seed germination (tab. 1). There were 2 exceptions here. 1) When the seeds were soaked for 30 minutes in 5% alcoholic solution of 0.4% camphor tree ethereal oil, the germination energy and capacity of the seeds decreased from 56.0% and 62.9% (check) to 40.0% and 54.3%, respectively. 2) When the seeds were soaked for 30 minutes in 5% alcoholic solution of 0.1% camphor tree ethereal oil solution, and then rinsed 3 times with sterilized water, their germination energy increased from 56% (check) to 63.7%. The number of abnormal seedlings occurred mostly in higher concentrations ethereal oil solutions treatments. Soaking seeds for 30 minutes in 5% alcohol of 0.4% camphor tree ethereal oil solution increased the amount of abnormal seedlings from 7.6% (check) to 18.3%. However, healthy ungerminated seeds, in majority of seed germination test results studied, were found in higher concentrations ethereal oil solutions treatments. In the best seed treatment, when they were soaked for 20 minutes in 5% alcoholic solution of 0.4% solution of it, and then soaked for 10 minutes in the distilled water, the percentage of healthy ungerminated seeds increased from 0.2% (check) to 9.0%. At the same time, soaking the seeds in the high concentration ethereal oil solutions resulted in decreasing the percentage of dead seeds. For example, dead seeds appeared the least (15.1%) after soaking in 5% alcoholic solution of 0.1% camphor tree ethereal oil for 30 minutes then rinsed 3 times with sterilized water (tab. 1).

Table 1. Effects of soaking lettuce 'Dumka' seeds in the camphor tree ethereal oil solutions of various concentrations on their germination

Tabela 1. Wpływ moczenia nasion sałaty odm. Dumka w roztworach olejku eterycznego z drzewa kamforowego o różnych stężeniach na ich kiełkowanie

Seed treatments Kombinacje nasion	Germination capacity Zdolność kiełkowania (%)	Energy of germination Energia kiełkowania (%)	Abnormal seedlings Siewki nienormalne (%)	Healthy ungerminated seeds Nasiona zdrowe niekiełkujące (%)	Dead seeds Nasiona martwe (%)
Check – Kontrola	62.90 ab*	56.01 bc	7.56 d	0.15 e	29.22 a
5A30	60.58 bc	52.33 bc	10.43 bcd	1.82 d	27.06 ab
0.1C30**	63.01 ab	55.34 bc	11.30 bcd	2.59 cd	22.95 abcd
0.1C30R***	70.41 a	63.71 a	8.93 cd	5.32 abc	15.13 e
0.1C20D10****	63.34 ab	59.67 ab	9.97 bcd	2.78 cd	23.31 abc
0.2C30	61.00 bc	50.00 c	14.21 ab	3.49 bcd	20.95 bcde
0.2C30R	66.20 ab	57.06 abc	12.66 bc	8.33 a	23.43 abc
0.2C20D10	62.39 abc	56.69 bc	7.23 d	6.30 ab	24.00 abc
0.4C30	54.33 c	39.99 d	18.30 a	8.95 a	18.32 cde
0.4C30R	62.59 abc	53.34 bc	13.06 abc	8.42 a	15.55 e
0.4C20D10	60.78 bc	52.78 bc	13.13 abc	8.97 a	16.47 de

*Means in a column followed by the same letters are not significantly different at $\alpha = 0.05$ level according to the Duncan's range test – średnie w kolumnie oznaczone tymi samymi literami nie różnią się od siebie istotnie według testu Duncana dla $\alpha = 0,05$.

**Soaked for 30 minutes in 5% alcoholic solution of 0.1% camphor tree ethereal oil – moczone przez 30 minut w 5% alkoholowym roztworze 0,1% olejku eterycznego z drzewa kamforowego.

*** Soaked in 5% alcoholic solution of 0.1% camphor tree ethereal oil for 30 minutes, and then rinsed with sterilized water 3 times – moczone w 5% alkoholowym roztworze 0,1% olejku kamforowego przez 30 minut, następnie płukane 3 razy w wodzie sterilizowanej.

****Soaked for 20 minutes in 5% alcoholic solution of 0.1% camphor tree ethereal oil, then soaked in distilled water for 10 minutes – moczone przez 20 minut w 5% alkoholowym roztworze 0,1% olejku eterycznego z drzewa kamforowego, następnie moczone przez 10 minut w wodzie destylowanej.

Seed germination after patchouli plant ethereal oil solutions treatments. None of the patchouli plant ethereal oil solutions soaking treatments affected germination of lettuce seeds (tab. 2). Neither of them affected the percentages of abnormal seedlings in the routine germination test. There was one exception here. When the seeds were soaked for 30 minutes in 5% alcoholic solution of 0.02% patchouli plant ethereal oil solution and then rinsed 3 times with sterilized water, their percentage of abnormal seedlings increased from 7.6% (check) to 11%. All used concentrations of the ethereal oil solutions increased the percentage of healthy ungerminated seeds in the routine germination test. In the best seed treatment, when seeds were soaked for 30 minutes in 5% alcoholic solution of 0.005% solution of the oil, the percentage of healthy ungerminated seeds increased from 0.2% (check) to 4.7%. Treating the seeds with various concentrations solutions of the patchouli plant ethereal oil had no effect on the percentage of dead seeds. The exception here was, when the seeds were soaked for 30 minutes in 5% alcoholic solution of 0.005% ethereal oil solution and then rinsed 3 times with sterilized water. The percentage of dead the seeds in that treatment decreased from 29.2% (check) to 20.0% (tab. 2).

Table 2. Effects of soaking lettuce 'Dumka' seeds in the patchouli plant ethereal oil solutions of various concentrations on their germination

Tabela 2. Wpływ moczenia nasion sałaty odm. Dumka w roztworach olejku eterycznego z paczuli o różnych stężeniach na ich kiełkowanie

Seed treatments Kombinacje nasion	Germination capacity Zdolność kiełkowania (%)	Energy of germination Energia kiełkowania (%)	Abnormal seedlings Siewki nienormalne (%)	Healthy ungerminated seeds Nasiona zdrowe niekiełkujące (%)	Dead seeds Nasiona martwe (%)
Check – Kontrola	62.90 ab*	56.01 ab	7.56 b	0.15 b	29.22 a
5A30	60.58 ab	52.33 ab	10.43 ab	1.82 a	27.06 ab
0.005P30**	65.67 ab	62.00 ab	7.95 ab	4.65 a	21.65 ab
0.005P30R	67.68 a	62.71 a	8.16 ab	3.89 a	20.00 b
0.005P20D10	61.08 ab	57.72 ab	9.26 ab	4.09 a	21.98 ab
0.01P30	65.04 ab	57.00 ab	10.91 ab	2.94 a	20.98 ab
0.01P30R	61.07 ab	47.54 b	10.60 ab	3.43 a	24.78 ab
0.01P20D10	63.40 ab	59.71 ab	8.27 ab	2.64 a	25.08 ab
0.02P30	59.06 b	49.67 ab	9.46 ab	2.85 a	28.18 ab
0.02P30R	61.67 ab	55.01 ab	11.00 a	4.35 a	22.52 ab
0.02P20D10	60.46 ab	51.66 ab	8.27 ab	3.72 a	26.75 ab

*For the explanations see table 1 – objaśnienia jak w tabeli 1.

**P – soaked in patchouli plant ethereal oil solution – P – moczone w roztworze olejku eterycznego z paczuli.

Table 3. Effects of soaking lettuce 'Dumka' seeds in camphor tree ethereal oil solutions of various concentrations on the seedlings' length

Tabela 3. Wpływ moczenia nasion sałaty odm. Dumka w roztworach olejku eterycznego z drzewa kamforowego o różnych stężeniach na długość siewek

Seed treatments Kombinacje nasion	Root (cm) Korzeń (cm)	Stem (cm) Łodyga (cm)
Check – Kontrola	2.67 ab*	2.21 ab
5A30	2.72 ab	1.97 cd
0.1C30	2.36 bcdef	1.75 e
0.1C30R	2.40 bcde	2.10 bc
0.1C20D10	2.78 a	2.27 a
0.2C30	2.00 f	1.48 f
0.2C30R	2.22 cdef	1.85 ed
0.2C20D10	2.13 def	1.93 d
0.4C30	2.09 ef	1.30 g
0.4C30R	2.48 abcd	1.74 e
0.4C20D10	2.54 abc	1.81 de

*For the explanations see table 1; objaśnienia jak w tabeli 1.

Seedlings' length after camphor tree ethereal oil solutions treatments. The conducted seedlings measurement showed that treating lettuce seeds with various solutions of camphor tree ethereal oil affected seedlings' length differently (tab. 3). In majority of cases studied, the seedlings were shortened. Seedlings with shorter roots mostly occurred in higher oil concentration solutions treatments. The shortest root (2.0 cm) of seedlings was found in the treatment in which the seeds were soaked for 30 minutes in 5% alcoholic solution of 0.2% ethereal oil solution. Regarding the length of stem, it was found that the higher the camphor tree ethereal oil concentrations, the shorter the length of stems. When the seeds were soaked for 30 minutes in 0.4% camphor tree ethereal oil solution, the length of stems was only 1.30 cm (tab. 3).

Seedlings' length after patchouli plant ethereal oil solutions treatments. The carried out experiment showed that the patchouli plant ethereal oil tested solutions had less effect on the length of the seedlings received from these seeds than the solutions of the camphor tree ethereal oil (tab. 3 and 4). In 2 cases studied, the seed treatment did elongate the seedlings' stems (tab. 4). However, at the same time, it clearly showed that the higher the ethereal oil concentrations solutions, the shorter the seedlings. Seedlings with the shortest roots (1.86 cm) were found in the treatment of soaking seeds for 30 minutes in 5% alcoholic solution of 0.02% ethereal oil solution. Similarly, seeds soaked in the same treatment had the shortest stems (1.57 cm).

Table 4. Effects of soaking lettuce 'Dumka' seeds in patchouli plant ethereal oil solutions of various concentrations on the seedlings' length

Tabela 4. Wpływ moczenia nasion salaty odm. Dumka w roztworach olejku eterycznego z paczuli o różnych stężeniach na długość siewek

Seed treatments Kombinacje nasion	Root (cm) Korzeń (cm)	Stem (cm) Łodyga (cm)
Check – Kontrola	2.67 ab*	2.21 cd
5A30	2.72 a	1.97 ef
0.005P30**	2.91 a	2.08 de
0.005P30R	2.86 a	2.49 a
0.005P20D10	2.34 bc	2.35 b
0.01P30	2.92 a	1.91 fg
0.01P30R	2.33 bc	2.03 ef
0.01P20D10	2.96 a	2.22 bc
0.02P30	1.86 d	1.57 h
0.02P30R	1.96 d	1.79 g
0.02P20D10	2.01 cd	1.94 ef

*For the explanations see table 1; objaśnienia jak w tabeli 1.

**P – soaked with patchouli plant ethereal oil solution; P – moczone w roztworze olejku eterycznego z paczuli.

Table 5. Effects of soaking lettuce 'Dumka' seeds in camphor tree ethereal oil solution of various concentrations on their total seed infestation and incidence of fungi

Tabela 5. Wpływ moczenia nasion salaty odm. Dumka w roztworach olejku eterycznego z drzewa kamforowego o różnych stężeniach na ich całkowite porażenie i zasiedlenie przez grzyby

Seed treatments Kombinacje nasion	Check Kontrola	5A30	0.1C30	0.1C30R	0.1C20 D10	0.2C30	0.2C30R	0.2C20 D10	0.4C30	0.4C30R	0.4C20 D10
<i>Alternaria alternata</i> (%)	99.60 a	95.81b*	82.42 c	76.35cd	74.15 cd	74.18 cd	70.16 d	80.12 c	48.51 e	54.54 e	57.01 e
<i>Cladosporium sphaerospermum</i> (%)	45.92 a	18.82 bc	24.01 b	11.43 cd	7.06 d	11.17 cd	15.4 bed	22.05 b	9.28 d	7.91 d	7.60 d
<i>Fusarium</i> spp. (%)	3.40 a	2.67 ab	0.90 ab	1.18 ab	0.20 b	0.40 ab	0.10 b	0.10 b	2.34 ab	0.92 ab	2.26 ab
<i>Ulocladium consortiale</i> (%)	0.59 a	1.74 a	0.76 a	2.53 a	0.40 a	0.10 a	0.59 a	0.10 a	0.40 a	0.40 a	0.10 a
<i>Verticillium</i> spp. (%)	0.10 ab	0.10 ab	0.10 ab	0 b	0.40 a	0 b	0 b	0 b	0 b	0 b	0 b
<i>Stemphylium botryosum</i> (%)	0.40 ab	0 b	0.40 ab	0 b	1.18 a	0 b	0.59 ab	0.10 ab	0.10 ab	0 b	0.10 ab
<i>Epicoecum purpurascen</i> (%)	0.10 a	0 a	0.10 a	0.20 a	0.90 a	0.59 a	0.10 a	0.40 a	0.40 a	0.40 a	0.10 a
<i>Gonatobotrys corda</i> (%)	5.52 a	0.20 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b
<i>Trichothecium</i> spp. (%)	1.41 a	0 b	0.10 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b
<i>Penicillium</i> spp. (%)	0 a	0.31 a	0 a	0.10 a	0.76 a	0.10 a	1.18 a	0 a	2.00 a	0.59 a	1.96 a
<i>Rhizopus</i> spp. (%)	0 b	0.40 ab	0 b	0 b	0.10 ab	0 b	0 b	0 b	1.00 a	0 b	0.10 ab
<i>Papulaspora</i> spp. (%)	0 a	0 a	0.10 a	0 a	0.10 a	0 a	0 a	0 a	0 a	0.31 a	0 a
No spore seeds (%)	2.11 de	0.10 e	4.31 cd	3.73 d	2.67 de	6.54 bcd	11.55 abc	11.37 abc	17.89 a	19.45 a	13.19 ab
Healthy seeds (%)	0 e	1.18 d	5.73 c	16.01 b	18.89 ab	15.92 b	15.30 b	7.89 c	25.85 a	23.88 a	24.41 a

*Means in a line followed by the same letters are not significantly different at $\alpha = 0.05$ level according to the Duncan's test. For further explanations, see table 1.

*Średnie w wierszu oznaczone tymi samymi literami nie różnią się od siebie istotnie według testu Duncana dla $\alpha = 0.05$. Objaśnienia jak w tabeli 1.

Table 6. Effects of soaking lettuce 'Dumka' seeds in patchouli plant ethereal oil solutions of various concentrations on their total seed infestation and incidence of fungi

Seed treatments Kombinacje nasion	Check Kontrola	5A30	0.005P30	0.005P 30R	0.005P20 D10	0.01P		0.02P		0.02P20	
						30	30R	30	30R	D10	D10
<i>Alternaria alternata</i> (%)	99.10 a	95.81 b*	94.87 bc	94.39 bc	93.19 bc	93.11 bc	92.58 bc	91.02 bcd	90.82 bcd	89.28 cd	84.92 d
<i>Cladosporium sphaerospermum</i> (%)	45.92 a	18.82 bc	15.72 bc	19.52 b	25.06 b	16.04 bc	17.15 bc	18.56 bc	8.75 c	19.75 b	18.28 bc
<i>Fusarium</i> spp. (%)	3.40 a	2.67 a	0 c	0.10 c	0 c	0.10 c	0.40 bc	0.40 bc	0.40 bc	2.34 ab	0.10 c
<i>Ulocladium consortiale</i> (%)	0.59 a	1.74 a	0.40 a	0.40 a	0.76 a	1.48 a	0.40 a	0.90 a	1.96 a	0.40 a	0.40 a
<i>Verticillium</i> spp. (%)	0.10 a	0.10 a	0.40 a	0 a	0.10 a	0 a	0 a	0 a	0 a	0 a	0.10 a
<i>Stemphylium botryosum</i> (%)	0.40 a	0 a	0.90 a	0.76 a	0 a	0.90 a	0.10 a	0.40 a	0.20 a	0.59 a	1.18 a
<i>Epicoccum purpurascens</i> (%)	0.10 a	0 a	0.59 a	0.10 a	0.10 a	0.10 a	0.10 a	0.40 a	0 a	0 a	0.10 a
<i>Gonatobotrys corda</i> (%)	5.52 a	0.20 c	0 c	0.10 c	2.50 ab	0.10 c	0 a	0.90 bc	0 c	0.10 c	0 c
<i>Trichothecium</i> spp. (%)	1.41 a	0 b	0 b	0 b	0 b	0 b	0.10 b	0 b	0 b	0 b	0 b
<i>Penicillium</i> spp. (%)	0 a	0.31 a	0 a	0.10 a	0.40 a	1.18 a	0.10 a	0.81 a	0 a	0.40 a	0.59 a
<i>Rhizopus</i> spp. (%)	0 c	0.40 abc	1.86 ab	2.67 a	3.12 a	0 a	0 a	0 a	0.40 abc	0.10 bc	0.10 bc
No spore (%)	2.11 ab	0.10 ab	1.48 ab	1.48 ab	2.78 a	1.18 ab	1.96 ab	1.41 ab	1.18 ab	0 b	0.40 ab
Healthy seeds (%)	0 c	1.18 bc	2.96 ab	3.29 ab	1.18 bc	4.38 ab	1.48 bc	4.01 ab	3.40 ab	3.32 ab	8.36 a

*For the explanations see tables 2 and 5.

*Objaśnienia jak w tabeli 2 i 5

Seed health after the camphor tree ethereal oil solutions treatments. Nine genera and species of fungi were identified in the check seeds (tab. 5). Thereinto, *Alternaria alternata* and *Cladosporium sphaerospermum* were the most common ones, around 99.6% *Alternaria alternata* occurred in the check seeds. All the other treatments showed differences in the presence of *Alternaria alternata* in comparison with the check seeds. With the increasing concentrations of camphor tree ethereal oil solutions, the percentages of seeds infested by *Alternaria alternata* declined. After soaking seeds for 30 minutes in 5% alcoholic solution of 0.4% camphor tree ethereal oil solution, the percentage of seeds with *Alternaria alternata* decreased by over 50%. Similar result was observed for *Cladosporium sphaerospermum*. When seeds were soaked for 30 minutes in 5% alcoholic solution of 0.4% solution of it, and then 3 times rinsed in the sterilized water, the percentage of seeds infested by *Cladosporium sphaerospermum* decreased by 38%. Camphor tree ethereal oil solutions treatments exerted partly impact on the occurrence of *Fusarium* spp. None spores were found on the surface of seeds for all treatments with significant difference. None healthy seed was received from the check one, whereas, a certain number of healthy seeds was obtained from various camphor tree ethereal oil solutions seed treatments, especially after soaking them for 30 minutes in 5% alcoholic solution of 0.4% camphor tree ethereal oil solution, most healthy seeds (25.9%) were found (tab. 5).

Seed health after patchouli plant ethereal oil solutions treatments. Differences were observed in the percentages of *Alternaria alternata* within the seeds of all the treatments (Table 6). The percentage of the seeds with infestation and incidence of it lowered when they were soaked in the patchouli plant ethereal oil solutions. In the best seed treatment, when the seeds were soaked for 20 minutes in 5% alcoholic solution of 0.02% patchouli plant ethereal oil solution and then soaked for 10 minutes in distilled water, the percentage of the seeds with *Alternaria alternata* lowered from 99.1% (check) to 84.9%. This lowering was observed in all tested seed treatments. Similarly, the patchouli plant ethereal oil solutions lowered the percentage of seeds infested by *Cladosporium sphaerospermum*. In the best seed treatment, when seeds were soaked for 30 minutes in 5% alcoholic solution of 0.02% patchouli plant ethereal oil solution, the percentage of seeds infested by this fungus lowered from 45.9% (check) to 8.8%. Very similar results, although on the much lower level, were recorded for *Fusarium* spp. and *Gonatotryps corda* fungi. Soaking seeds in the patchouli plant ethereal oil solutions lowered the percentage of seeds, in which both fungi were found. The used camphor tree ethereal oil solutions in higher concentrations increased the percentage of seeds with no spores. In the best treatment, when the seeds were soaked for 30 minutes in 0.4% solution of it without rinsing, the percentage of seeds with no spores increased from 2.1% (check) to 19.5%. No such differences were found for the patchouli plant ethereal oil solutions (tab. 5 and 6). Healthy seeds, finally, were detected in all treatments except the check seeds. It was, also, of obvious difference within all the treatments. After soaking the seeds for 20 minutes in 5% alcoholic solution of 0.02% patchouli plant ethereal oil solution, and then soaking in distilled water for 10 minutes, 8.4% healthy seeds were identified (tab. 6).

DISCUSSION

Many factors have been considered affecting seed germination. Seed moisture content is one of the major factors of them. If seeds are dried out, then water has little or no mobility, which may increase the rate of membrane deterioration; while if seed moisture content is too high, the seeds lose viability. The optimal seed moisture content for vegetable seeds is 6–14% [Benech-Arnold and Sánchez 2004]. Nevertheless, in the carried out experiment, moisture content of tested lettuce seeds was 5.5%. Notably, too low seed moisture content might be one of the reasons for their low germination capacity.

Accordingly, germination energy reflects germination rate, uniformity, vigour and viability [Bewley et al. 2006]. Theoretically, no seeds in a population are immune from the effects of aging. Thus, after a given period of storage, even the shortest lived individuals in a population are still capable of germination in a certain percent. Therefore, seeds in conducted experiments with low germination energy might have experienced deterioration process.

Germination, according to the Association of Official Seed Analysts (AOSA), is “the emergence and development from the seed embryo of those essential structures which, for the kinds of seed in question, are indicative of the ability to produce a normal plant under favourable conditions”. Liu et al. [2006] confirmed that camphor tree ethereal oil was significantly toxic to wheat grains at 500 µg/ml. Kaborek et al. [2006] came to similar conclusions. They proved that ethereal oils of *Citrus citratus* and *C. giganteus* were slightly phytotoxic to seeds. In the conducted experiment, there is no doubt that high concentration oil, when not removed after treatments, was mildly phytotoxic to the seeds. Besides, chemical interfere may occur in seed metabolism. Abdul-Baki [1974] discovered that seeds sterilized substance sodium hypochlorite reduced the concentration of amino acids in seeds by reacting with them. The reaction also resulted in high production of CO₂ and low uptake of amino acids by seeds. The carried out experiment did not bring any convincing proof that the used solutions of both ethereal oils were toxic to seeds, which then was expressed by lowering their germination. The exception here was the camphor tree ethereal oil used at the highest concentration with subsequent no rinsing, in which both energy and capacity of germination were lowered.

Ethanol accumulation has been proved to be one of the possibilities to restrain seed germination. However, from the methods' points of view, it was necessary to put in these experiments one seed treatment with soaking the seeds only in the alcoholic solution. Moreover, Crawford [1977] suggested that all germinated seeds undergo a period of anoxia after imbibition and before rupture of the testa. Due to the fact that toxic effect of ethanol itself was not a purpose of these experiments and gave no clear differences and thereafter was not supported by any further physiological measurements, hence, no conclusion here can be proposed.

The action of microorganisms, viruses and nematodes can markedly be the factors affecting seed germination and vigour, in terms of germination energy. Field fungi such as *Fusarium*, *Cladosporium*, *Nigrospora*, *Curvularia* and *Alternaria* are often associated with seeds at harvest, indicated by discoloration, shrivelling or damage to the embryo, and may be caused by the invasion of these types of fungi [Basra 1995]. In the conducted experiments, *Alternaria alternata*, mostly, if not all, infested both treated and

untreated seeds. The fungus develops well at a wide range of temperatures, even in a refrigerator. Therefore, even when the tested lettuce seeds were stored in a fridge, they were not immune to *Alternaria alternata*. Moreover, the fungus may spread into and rot tissues internally with a mat of mycelium that is white at first but later turns brown to black forms on the surface of the rotted area [Agrios 2005]. In the experiment, most of the dead seeds that performed black forms could be rotted by *Alternaria alternata* (fig. 1). The seedborne fungus, after being treated with both tested ethereal oils, was suppressed effectively. Moreover, the carried out experiments proved that both ethereal oils had a strong disinfection effect on the tested lettuce seeds. This resulted in lowering the number of dead seeds and high increase of healthy ungerminated seeds. In the conducted experiment, *Cladosporium sphaerospermum* was almost detected from dead seeds but not from abnormal seedlings. However, Subbarao [2005] working with bean and lettuce, showed that *Cladosporium* sp. affected neither the growth of bean or lettuce nor caused any disease on these crops.



Fig. 1. Black dead lettuce seeds (marked with an arrow) in germination test in the laboratory
Ryc. 1. Czarne martwe nasiona sałaty (zaznaczone strzałką) w ocenie kiełkowania w laboratorium

Ren and He [2008], working with lettuce, confirmed that under the stress of sodium chloride, the growth of stem was inhibited. Sodium chloride, which is osmotic, exerted negative impact on lettuce stem growth. The applied camphor and patchouli plant ethereal oils in the conducted experiments could have produced stress as well to the growth of stem via permeability in a similar way. Thus, further research should be continued to seek out those phenomena.

In germination test, the incidence of the percentage of healthy ungerminated seeds between check and treated ones exhibited that both camphor tree and patchouli plant ethereal oils carried antifungal properties. The number of dead seeds, between ethereal

oils treatments and the check one, as well, can again prove this trait. Similar conclusions were achieved previously [Zhang et al. 2002, Mo et al. 2004, Wang 2007]. Therefore, it can not be denied that both camphor tree and patchouli plant ethereal oils played positive roles in antifungal actions to improve seed quality in this way. However, there is another, more general question. How come in the check seeds, 1/3 of them did not germinate? Could this be related to dying of the seeds in germination caused by bacteria or any individual fungus? This process must have gone rapidly, because after treating them with the tested ethereal oils, they changed from dead seeds into healthy ungerminated ones. What happened in that short time in the seeds? The answer to this question is still unknown and needs further research.

CONCLUSION

1. The tested ethereal oils had no effect on the germination of lettuce seeds however soaking the seeds in the ethereal oils resulted in decreasing the percentage of dead seeds in the tested samples.

2. Soaking the seeds in the ethereal oils solutions resulted in increasing of percentage of healthy ungerminated seeds.

3. Use of high concentrations of ethereal oils had no effect of the lettuce seeds germination. Soaking the seeds in higher concentrations solutions of the ethereal oils resulted in shortening the length of the seedlings received from them.

4. The used camphor tree ethereal oil lowered the amount of identified *Alternaria alternata* and *Cladosporium sphaerospermum* fungi on the seeds. The highest concentration of the used ethereal oil was the most effective.

5. Soaking the lettuce seeds in camphor tree and patchouli plant ethereal oils solutions increased their seed quality through decreasing the number of dead seeds and increasing the number of healthy ungerminated seeds in seed testing.

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**WPLYW TRAKTOWANIA NASION SAŁATY (*Lactuca sativa* L.)
ROZTWORAMI OLEJKÓW ETERYCZNYCH Z DRZEWA
KAMFOROWEGO (*Cinnamomum camphora* L.) I PACZULI (*Pogostemon
cablin* Benth.) NA ICH KIELKOWANIE**

Streszczenie. Jednym z najważniejszych problemów nasiennictwa ogrodniczego dziś jest poszukiwanie nowych metod poprawy jakości nasion. Szczególnie interesujące są te z nich, w których wykorzystuje się substancje naturalne. Jedną z takich grup są olejki eteryczne z roślin. Nasiona handlowe sałaty (*Lactuca sativa* L.) odmiany Dumka potraktowano na 9 różnych sposobów 5% alkoholowym roztworem olejku eterycznego z drzewa

kamforowego (*Cinnamomum camphora* L.) i na 9 różnych sposobów 5% alkoholowym roztworem olejku eterycznego z paczuli (*Pogostemon cablin* Benth.). Następnie nasiona poddano rutynowej ocenie kiełkowania, zmierzono długość otrzymanych siewek i oceniono zdrowotność nasion. Badane olejki eteryczne nie wpłynęły na kiełkowanie nasion sałaty, natomiast zmniejszyły odsetek martwych nasion w badanych próbach. Moczenie nasion w badanych roztworach olejków zwiększyło także odsetek nasion zdrowych niekiełkujących. Traktowanie nasion roztworami olejków eterycznych o wyższych stężeniach, choć nie wpłynęło na ich kiełkowanie, to jednak skróciło długość otrzymanych z nich siewek. Ponadto stosowane olejki eteryczne obniżyły ilość oznaczonych na nasionach grzybów: *Alternaria alternata* i *Cladosporium sphaerospermum*.

Słowa kluczowe: moczenie nasion, jakość nasion, zdolność kiełkowania, zasiedlenie i porażenie nasion przez grzyby

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