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THE EFFECT OF TREATING THE SEEDS OF *Brassica oleracea* L. WITH AQUEOUS EXTRACTS ON THE GERMINATION CAPACITY AND SEED HEALTHINESS

Summary

In the experimental trial the influence of the aqueous extracts on the germination capacity and surface contamination of the seeds of Brassica oleracea L. white cabbage, 'Stonehead' variety was assessed. The extracts in the form of macerate, infusion and decoction were prepared with the use of different morphological parts of 40 plants species. The results obtained allowed to indicate the plants the extracts of which both stimulated the germination of the seeds and limited their surface contamination with microorganisms. Additionally, the information which manner of obtaining the extracts stimulated the viability and health of analyzed seeds most effectively was received. Among applied extracts the germination capacity of cabbage seeds was stimulated the most effectively by preparations of Juniperus communis fruit (+20,67%), Verbascum thapsiforme flowers (+20,48%), and green parts of Artemisia absinthium (+19,61%). In turn, the healthiness of the cabbage seeds provided the greatest degree by treatment all forms of extracts (macerate, infusion, decoction) of Carum carvi fruit (-78,14%), roots of Archangelica officinalis (-72,48%) and Salix alba and S. purpurea bark (-69,03%).

The germination of seeds was stimulated by the extracts in the form of infusions, whereas the microorganisms colonization was limited by macerates.

Key words: seeds, cabbage, plant extracts, germination capacity, surface contamination, seeds healthiness

WPŁYW ZAPRAWIANIA WYCIĄGAMI ROŚLINNYMI NA ZDOLNOŚĆ KIEŁKOWANIA I ZDROWOTNOŚĆ NASION Brassica oleracea L.

Streszczenie

W doświadczeniu oceniano wpływ działania wyciągów wodnych na zdolność kiełkowania oraz kontaminację powierzchniową nasion kapusty białej Brassica oleracea L. odmiany 'Kamienna głowa'. Wyciągi w postaci maceratów, naparów i wywarów wykonano z różnych części morfologicznych 40 gatunków roślin. Uzyskane wyniki badań przyczyniły się do wskazania roślin, z których wyciągi działały zarówno stymulująco na kiełkowanie nasion jak i ich zdrowotność. Dodatkowo uzyskano informację, który ze sposobów pozyskiwania wyciągów najsilniej stymulował zdolność kiełkowania i zdrowotność badanych nasion. Spośród zastosowanych wyciągów zdolność kiełkowania nasion kapusty najkorzystniej stymulowały preparaty z owoców Juniperus communis (+20,67%), z kwiatów Verbascum thapsiforme (+20,48%), oraz z części zielonych Artemisia absinthium (+19,61%). Z kolei zdrowotność nasion kapusty w największym stopniu zapewniało zaprawianie nasion każdą z wykorzystanych form wyciągów (macerat, napar, wywar) z owoców Carum carvi (-78,14%), korzeni Archangelica officinalis (-72,48%) oraz kory Salix alba i S. purpurea (-69,03%). Kiełkowanie nasion stymulowały wyciągi w formie naparów, natomiast zasiedlenie drobnoustrojami ograniczały maceraty.

Słowa kluczowe: nasiona, kapusta, wyciągi roślinne, zdolność kielkowania, kontaminacja powierzchniowa, zdrowotność nasion

1. Introduction

The customers' interest in healthy food causes increased demand for raw material coming from ecological farming. Such raw material must have a certificate, which, at the same time, is the confirmation that the plant protection preparations included in the list of the Institute of Plant Protection (www.ior.poznan.pl) were applied. In the ecological agriculture it is also admitted to apply the preparations of natural origin, provided so as to stimulate the growth of crop and limit its colonization by pathogens. The appropriately selected plant extracts eliminate only agrophags, without destroying the useful organisms, whereas, for instance, the preparations for seeds treatment influence advantageously their germination capacity and the developing plants have longer root system, are more green and have bigger mass [16].

The analysis of the plant extracts documented the antibacterial and antifungal effects, however, there are a few complex *in vitro* studies of the effects inflicted by the products of plant's metabolism on agrophags. This results probably from the difficulties found in the normalized methods of their sensibility assessment [11, 13, 15, 16].

The impact of the preparations of plant origin on the environment is also of significance. The chemical compounds in their powdery form increase the area of their spread to the areas adjacent to the crop fields and thus influence adversely the biodiversity. In case of the preparations of plant origin they are subject to biodegradation quicker than the synthetic compounds [12].

The main purpose of the conducted research was to define the influence of the aqueous extracts from 40 different plant species on the germination capacity of cauliflower seeds which had been treated therewith. The selection of plant species was dictated by previous studies that demonstrated that the analyzed plant extracts stimulated the healthiness and germination capacity of oily, cereal and legume plants. The measurable effect of the paper was, however, indicating of the plants which, apart from huge fungistatic potential stimulated the germination of cabbage seeds. The applying the extracts from these plants in the ecological farming and in the integrated farming may be an alternative for chemical measures of plant protection or may be the measure supporting the protection of plants grown conventionally.

2. Material and research methods

The research material included not-treated seeds of white cabbage *Brassica oleracea L*. 'Stonehead' variety, coming from Zakład Hodowlano-Nasienny (Farming and Seed Plant) in Gołębiewo.

The plants from which the extracts in the form of macerate, infusions and decoction were prepared were listed in Table 1. The dried plant material came from the 'Herbapol' store.

Plant extracts in the form of macerate, infusion and decoction were prepared following the below presented methodology. Macerate - 5 g of dried plant were poured with 100 ml of cold water and left for 24 h in temperature of 20°C, and afterwards were filtered; infusion - 5 g of plant were weighed and poured with 250 ml of boiling water and left under cover for 30 minutes, when cold filtered. Decoction - 8,75 g of each dried plant was weighted and poured with one liter of distilled water. The suspension was thoroughly stirred and left for 24 hours and then boiled for 15 minutes. Boiled decoctions were sieved on the sieve with gauze lining into glass containers and analysed when cooled [23].

The plant extract obtained after the filtration were used for wet treatment of the seeds. The seeds were shaken in the laboratory shaker of 358 A type for 10 minutes. The seeds in the extracts for treating were left for 20 hours in the temperature of 21°C, covered with aluminum foil [16].

The trial was carried out with the use of tissue paper test (ISTA 2007 – International Seed Testing Association – Chapter 7) determining: germination capacity. The assess-

ment criterion referred to the number of seeds: germinating normally, germinating abnormally; healthy not germinating and dead seeds (colonized by bacteria and fungi). The study was performed in 3 replicates for each type of extracts of each herb and a control sample. For every repetition accounted for 100 seeds of cabbage.

The obtained results were calculated as a percentage of the control, which was white cabbage seeds treated with sterile water.

The formula used to calculate the% of seed compared to the control:

$$X \% = (100 \times a/k) - 100$$

where: X% - deviation from the control combination, a average number of seeds in the test sample, k - average number of seeds in the control sample. In the case of germination capacity, the values with the (+) mark indicate the increase in germination relative to the control. Whereas in the case of the healthiness of the seeds the values marked with the sign (-) have proven to reduce the contamination of the seed by the microorganisms in relation to the control.

The obtained results were statistically developed by the analysis of variance (ANOVA) with single classification (P = 95%), for plant species, the manner of extract preparation and assessment criterion (two-factor analysis of variance). The least significant difference (Fisher test - NIR_{0.05}) was also determined. The results calculated into per cents in relation to the control object which were the cauliflower seeds treated with sterile water were presented in Tables 2 and 3. To compare the results obtained for the cabbage seeds and the assessment criteria (between germination capacity and seeds healthiness) the correlation ratio r (for $\alpha =$ 0,05 and for $\alpha = 0,01$) and variable V% were used. The significance of the correlation ratio at P=95% was marked with the sign "*" and at P = 99% by a double sign "**".Statistical analysis was carried out with the use of the following software ANW (Analysis of Experience Variance) and ANK (Analysis of Experience Correlation).

Tab. 1. Plants and organs from which aqueous extracts were prepared *Tab. 1. Rośliny, z których zostały przygotowane wyciągi*

1. Acorus calamus L. (rhizome);	21. Lavandula vera L. (flowers)
2. Aesculus hippocastanum L. (bark)	22. Levisticum officinale L. (roots)
3. Aesculus hippocastanum L.(flowers)	23. Linum usitatissimum L. (seeds)
4. Allium sativum L.(bulb)	24. Marrubium vulgare L. (herb)
5. Archangelica officinalis Hoffm. (roots)	25. Matricaria chamomilla L. (inflorescence)
6. Arctium lappa L. (roots)	26. Melissa officinalis L.(leaves)
7. Artemisia absinthium L. (herb)	27. Mentha piperita L. (leaves)
8. Artemisia vulgaris L. (herb)	28. Origanum majorana L. (herb)
9. Betula verrucosa Ehrh. (leaves;)	29. Pinus sylvestris L. (young sprouts)
10. Calendula officinalis L. (flowers)	30. Quercus robur L. (bark)
11. Camelina sinensis L. (leaves)	31. Ribes nigrum L. (leaves)
12. Carum carvi L. (fruit)	32. Rosa canina L.(fruit)
13. Coriandrum sativum L. (fruit)	33. Salix alba and S. purpurea L.(bark)
14. Crataegus oxyacantha L. (flowers)	34. Sambucus nigra L. (flowers)
15. Equisetum arvense L. (herb)	35. Saponaria officinalis L. (roots)
16. Frangula alnus Mill. (bark)	36. Satureja hortensis L. (herb)
17. Hyssopus officinalis L. (herb)	37. Taraxacum officinale Web. (roots)
18. Inula helenium L. (roots)	38. Urtica dioica L. (leaves)
19. Juglans regia L. (leaves)	39. Verbascum thapsiforme L. (flowers)
20. Juniperus communis L. (fruit)	40. Zea mays L. (stigmas)

Source: own work / Zródło: opracowanie własne

Tab. 2. Germination capacity of white cabbage seeds (deviation from control, %) depending on plant species from which
extracts were prepared and method of their preparation

Tab. 2. Zdolność kiełkowania nasion kapusty białej (odchylenie od próbki kontrolnej, %) w zależności od gatunku rośliny, z której wyciągi były zrobione oraz od sposobu ich przygotowania

	F	Form of plant extracts			
Species of the plant from which the extracts were prepared	macerate	decoction	brew	Medium	
Acorus calamus	5,57	-2,09	-2,31	0,39	
Aesculus hippocastanum	-14,29	-13,59	-12,54	-13,47	
Aesculus hippocastanum	3,82	2,79	-8,91	-0,77	
Allium sativum	-0,35	-3,83	-9,90	-4,69	
Archangelica officinalis	3,48	9,41	20,13	11,01	
Arctium lappa	1,04	-1,74	4,95	1,42	
Artemisia absinthium	34,84	23,34	0,66	19,61	
Artemisia vulgaris	1,04	-2,79	-15,18	-5,64	
Betula verrucosa	-1,04	-20,56	-43,89	-21,83	
Calendula officinalis	-3,83	-2,79	2,31	-1,44	
Camelina sinensis	-5,57	-6,27	-9,24	-7,03	
Carum carvi	-21,95	20,91	20,13	6,36	
Coriandrum sativum	3,83	3,83	-7,26	0,13	
Crataegus oxyacantha	7,66	2,44	-1,65	2,82	
Equisetum arvense	4,18	0,35	-30,69	-8,72	
Frangula alnus	0,00	1,74	-12,21	-3,49	
Hyssopus officinalis	12,89	21,25	7,92	14,02	
Inula helenium	20,91	10,45	-16,50	4,95	
Juglans regia	1,04	4,18	-4,62	0,20	
Juniperus communis	28,92	20,21	12,87	20,67	
Lavandula vera	-7,66	-6,97	-13,20	-9,28	
Levisticum officinale	-3,48	8,36	4,62	3,17	
Linum usitatissimum	5,23	3,83	-15,84	-2,26	
Marrubium vulgare	-20,56	5,23	-11,22	-8,85	
Matricaria chamomilla	17,77	26,83	-18,15	8,82	
Melissa officinalis	20,91	25,44	-1,65	14,90	
Mentha piperita	-6,27	0,00	-16,17	-7,48	
Origanum majorana	1,74	5,92	21,78	9,81	
Pinus sylvestris	-16,38	0,00	-7,26	-7,88	
Quercus robur	-0,35	1,74	-3,30	-0,64	
Z Ribes nigrum	13,94	5,57	25,08	14,86	
Rosa canina	-2,79	16,72	-7,92	2,00	
Salix alba i S. purpurea	-7,66	-1,39	20,13	3,69	
Sambucus nigra	2,44	-12,19	-15,84	-8,53	
Saponaria officinalis	5,23	2,44	-12,21	-1,51	
Satureja hortensis	-8,71	5,92	4,29	0,50	
Taraxacum officinale	28,57	21,60	-6,93	14,41	
Urtica dioica	-6,62	1,04	-7,59	-4,39	
Verbascum thapsiforme	21,95	23,00	16,50	20,48	
Zea mays	5,92	2,79	-9,90	-0,40	
Medium	3,14	5,08	-4,02	1,40	
	lant species = 5,64	- , ~ ~	$NIR_{interaction} = 9,$		

Source: own work / Źródło: opracowanie własne

3. Results and discussion

The analysis of variance indicated that both germination capacity and seeds healthiness change depending on the origin of the extract (plant species used to prepare the extract) as well as the manner of its preparing. The interactions of 1st degree also proved to be significant.

Treatment of white cabbage seeds with the extracts resulted in average increase in the germination capacity by +1,4%. The extracts prepared from different plant species showed different influence on the germination capacity, irrespectively the manner of their preparing.

Germination capacity was stimulated by the extracts from 52,5% of plants (range from +0,12 to +20,52%). The most advantageous influence on germination capacity was

noted for following extracts: from *Juniperus communis* fruit (average germination capacity of 20,52%), from *Verbascum thapsiforme* flowers (20,42%) and from green parts of *Artemisia absinthium* (19,28%). However, some extracts used in the trial inhibited the seeds germination when compared to control. The germination capacity was limited by 47,5% of extracts (range from -0,56% to -22,24%). The extract from *Betula verrucosa* leaves (-22,24%), from *Aesculus hippocastanum* bark (-13,45%), from *Lavandula vera* flowers (-9,35%) limited the germination capacity the most. In spite of differences in the strength of extracts influencing the germination capacity ranking thereof, their activity taken into account was in fact compliant (r > limiting r).

Also, the manner of extracts preparing, irrespective the remaining assessed factors (tab. 2) differentiated the seeds

germination capacity. Infusion (+5,08%) and macerate (+3,14%) influenced it the most stimulating, whereas the decoction limited it by -4,02%.

In spite of different activity of aqueous extracts it was found out that their influence on the germination capacity of treated seeds of white cabbage was in fact compliant. The value of correlation ration amounted to $r = 0.73^{**}$.

It was also ascertained that irrespective of the extract origin the reaction of tested cabbage seeds depended upon the manner of their preparing.

Analysing the reaction of cabbage seeds to the aqueous extracts (tab.2), depending on their origin and manner of their preparing (1^{st} degree interaction) it was ascertained that among 120 tested combinations, in 55% of cases there was a

significant increase in the germination capacity (when compared to absolute control combination), and in 45% its drop.

Comparing the reaction of cabbage seeds to the extracts used for the treating thereof it was found out that the germination capacity was favourably influenced, to a smaller or bigger degree, by all forms of extracts (macerate, infusion, decoction) prepared from *Juniperus communis* fruit (average: macerate +28,92%, infusion +20,21%, decoction +12,81%). Similar influence was observed in case of extracts from *Verbascum thapsiforme* flowers (average: macerate +21,95%, infusion +23,00%, decoction +16,50%) and from green parts of *Artemisia absinthium* (macerate +34,84%, infusion +23,34%, decoction +0,66%).

Tab. 3. The number of contaminated seeds of white cabbage (deviation from control, %) depending on plant species from which extracts were prepared and method of their preparation

Tab. 3. Liczba zarażonych nasion białej kapusty (odchylenie od próbki kontrolnej, %) w zależności od rodzaju rośliny, z której wyciągi zostały sporządzone oraz od sposobu ich przygotowania

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Source: own work / Źródło: opracowanie własne

The seeds germination capacity was influenced most favourably by the extracts prepared from 72,5% of analysed plants (range +0,35% to +26,83%), and in particular infusions prepared from capitulum of Matricaria chamomilla (average germination capacity +26,83%), from Melissa officinalis leaves (average germination capacity +25,44%), from green parts of Artemisia absinthium (average germination capacity +23,34%). Macerate prepared from 60% of plants (range +1,04% to +34,84%) stimulated the germination capacity. The best when they were prepared from green parts of Artemisia absinthium (+34,84%), fruits of Juniperus communis (+28,92%) and roots of Taraxacum officinale (+28,57%). But, the infusion made of 32,5% of analysed plant species (range +0,66% to +25,08%) influenced effectively the germination capacity. The most effective proved to be the infusions of Ribes nigrum leaves (+25,08%), green parts of Origanum Majorana (+21,78%) and the roots of Archangelica officinalis, fruit of Carum carvi and bark of Salix alba and S. purpurea (germination capacity +20,13% in relation to control).

The reaction changeability (V%) expressed by the germination capacity amounted to 9,73%.

Treatment of white cabbage seeds with the plant extracts caused the number of germinating seeds colonized by microorganisms drop by average of -26,53% in relation to control.

The number of seeds colonized by microorganismns was limited by 90% of extracts (from -3,43% to -80,59%), and in particular by extracts made of the fruits of *Carum carvi* (-80,59%), roots of *Archangelica officinalis* (-74,83%), and green parts of *Hyssopus officinalis* (-70,85%).

Treatment of the seeds with the extracts from only 10% of herbs caused the increase of the number of seeds with the symptoms of being colonized by microorganisms (from + 1,71% to + 5,14%). And the increase was the biggest when the extracts were made of *Frangula alnus* bark (+5,14%), leaves of *Betula verrucosa* (+ 4,59%) and green parts of *Marrubium vulgare* (+2,33%).

Amongst the analysed aqueous extracts, the number of the seeds showing the symptoms of contamination with bacteria and fungi was limited both by macerate (average drop by -57,46% in relation to control), and the infusion (-44,35%). Only in case of decoction the increase of contamination was observed (+22,23%).

The reaction changeability (V%) determined by the number of seeds at which the colonization by microorganisms was ascertained amounted to 23,58%.

Treatment of the cabbage seeds with infusions made of 92,5% of plants caused the drop of their contamination in the range from -8,70% to -84,06%. The biggest reduction was observed after treating the cabbage seeds with the infusions made of green parts of *Artemisia absinthium* (-84,06%) and *Hyssopus officinalis* (-84,06%), as well as fruit of *Carum carvi* (-81,16%).

Macerate made of 97,5% of plants influenced the decrease of number of colonized seeds (range from -1,45% to – 88,41%). The biggest reduction was ascertained when macerate from *Carum carvi* fruit (-88,41%), green parts of *Artemisia absinthium* (-86,96%) and *Hyssopus officinalis* (-86,96%) were used.

The healthiness of cabbage seeds was stimulated in result of the application of all forms of extracts (macerate, infusion, decoction) made of fruit of *Carum carvi*, bark of *Salix alba* and *S. purpurea*, as well as roots of *Archangelica officinalis*. It was ascertained that 76% from among 120 of analyzed combinations significantly limited the number of colonized seeds when compared to absolute control combination and 24% caused its increase.

Colonization of the cabbage seeds increased mostly after treating by the extracts of *Linum usitatissimum* seeds (+191,90%), extracts from *Betula verrucosa* leaves (+110,80%), and extracts from roots of *Acorus calamus* (+94,59%).

Analysis of germination capacity variation of the seeds treated with the extracts made of different plants and the number of seeds with the symptoms of microorganisms colonization proved that the stronger the extracts limited the microorganisms contamination of the seeds the better was their germination. This is confirmed by correlation ratio (contamination x capacity), which amounted to -0,68**.

Many species of plants and different parts thereof are applied in folk medicine, herbal treatment and in cosmetic industry but only within last few years they started to be recognized as a potential source of antimicrobials, which could be used in the plant protection [1-7].

The products of plant metabolism are created in the transformation processes of shikimic, mevalonic and malonic acids. The synthesis of created compounds is influenced by: stage of growth and development of the plant, genetic conditions and conditions of the environment. The last one include light availability - the intensity of UV radiation, temperature, and also the availability of nourishment and mineral products. Also the time of the year, day and other external factors determine the concentration of the above mentioned substances. Preparation in the form of aqueous extracts is the most common and the simplest method making use of beneficial effects of herbs. The factor determining their activity is the temperature of extraction process. Depending on the temperature transferring from the compounds biologically active to extracts is differentiated as they differ in their volatility, molecular weight and chemical structure [14].

The tests using the aqueous extracts to treat crop seeds were conducted in the largest scope by Sas-Piotrowska and others. In these analysis the significant variation of the influence of extracts made of different plants and different manner of their preparation onto the germination capacity and the contamination of the seeds by microorganisms was ascertained. At the same time, the significance of interaction depending on the extract origin (herb), manner of the preparation thereof, as well as interaction between these factors was ascertained [16-22]. Also, previous analysis of the authors of this paper conducted on, among others, the seeds of oil crops, root crops and bean family confirmed that both seeds viability (energy and germination capacity) and the contamination with microorganisms change depending on the origin of the aqueous extract (species of herb) and the manner of its preparation (macerate, infusion, decoction). It was also ascertained that the aqueous extracts limited the number of seeds colonized by bacteria and fungi to different degree, in particular the manner of extract preparation differentiated the seeds colonization. In case of the assessment of health and viability of the seeds and their treatment by aqueous extracts it was ascertained that, for instance in case of beet, infusions were the most favourable [5].

Obtained results of own research and those of presented authors indicate that the use of herbs of large content of secondary metabolism products which are successfully used by herbal treatment may become an alternative for chemical preparations of plant protection. The results of own research and those obtained by Sas-Piotrowska and Piotrowski [22], and also earlier results of Czerwińska and others [5] confirm biological activity of plant extracts. However, it is dependent upon several factors, the most important of which is the content of defined chemical compounds and their diffusion ability. It was observed in different influence of macerate, infusion and decoction made from the same plant species. This probably results from possible losses caused by solvent evaporation during their preparation and different solvability of active compounds contained in plants [22].

Analyzing the results of own research and comparing them with the results obtained by other authors it must be underlined that, in spite of large differences of the influence of aqueous extract on seeds germination it was observed that the stronger the applied extracts influence the contamination of seeds the better was their germination. Thus, preparing the extracts from these plants and applying them to treat the seeds of crop may become the method supporting the protection of plants against agrophags. The more so, these are very common plants in our environment and different parts thereof assist the treatment in many conditions.

4. Conclusions

1. Germination capacity of cabbage seeds and the number of sick seeds change depending upon the extract origin (species of the plant the extract was prepared from) and the manner of its preparation.

2. Germination capacity was stimulated by extracts from 52,5% of plants. Infusions influenced the germination capacity the most favourably. Amongst the plant species the ones made from fruit of *Juniperus communis* (+20,67%), from flowers of *Verbascum thapsiforme* (+20,48%) and from green parts of *Artemisia absinthium* (+19,61%) proved to be the best.

3. The number of sick seeds was limited by 90% of extracts, and in particular by extracts made of fruit of *Carum carvi* (-78,14%), roots of *Archangelica officinalis* (-72,48%) and bark from *Salix alba and S. purpurea* (-69,03%).

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