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Evaluation of Germination of Soybeans Treated with Natural Environment-Friendly Extracts Depending on the Method of their Application

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1. Introduction

The growing interest in the production of legumes, including soybean, is partly caused by integrated crop protection principles introduced into Polish agriculture, an important element of which is the rational succession of cultivated species, limiting the occurrence of harmful organisms (Ehler 2006). Legumes plants are considered a valuable link in crop rotation, not only because of nitrogen enrichment (Bagayoko 2000) but also their structure- building effect. Some varieties of legumes, including soybean, can be included in crop rotation to suppress pathogens, e.g. pathogenic root nematodes (Rodríguez-Kábana et al. 1988). The promotion of non-chemical methods in plant protection has led to the search for new, environmentally safe natural substances, referred to as biopesticides or biopreparations (Chandler et al. 2011, Kocira et al. 2017a-b, Procházka et al. 2017, Kocira et al. 2018a-b, Czerwińska & Szparaga 2015). Some of them can also be used in organic farming. One of the most important problems of organic farming is the shortage of natural seed treatments that are permissible for practical application (Rochalska et al. 2010). Lack of sufficient protection of seeds in the initial stages of development results in an incidence increase of seedlings for soil diseases. This leads to the weakening of seed sowing value and yield reduction (Shafique et al. 2016). Insufficient quality and organic seed salubrity encourages to search for alternative and natural substances used as seed treatments (Orzeszko- Rywka et

al. 2011). The aim of the conducted research was to show the influence of natural water extracts prepared on the basis of *Aesculus hippocastanum* L. on the shooting and germination of three varieties of soybean depending on the method of their application.

2. Material and methods

2.2. The greenhouse experiment

The seeds of three varieties of soybean (*Glycine max* L.) were used in the experiment: Abelina, Augusta and Merlin. The seeds were treated with aqueous extracts prepared from flowers *Aesculus hippocastanum* L. in dried from. Three types of aqueous herbal extracts were prepared: macerate, infusion, decoction, according to the procedure used by Sas-Piotrowska et al. (2005), Sas-Piotrowska and Piotrowski (2011). Decoction – 7 g of each dried plant was weighted and poured with 750 mm of distilled water. The suspension was thoroughly stirred and left for 24 hours and then boiled for 15 minutes. Macerate – 17,5 g of dried plant were poured with 350 ml of cold water and left for 24 h in temperature of 20°C, and afterwards were filtered. Infusion – 7 g of plant were weighed and poured with 350 ml of boiling water and left under cover for 30 minutes, when cold filtered. The three- factorial experiment was carried out in summer, in a greenhouse (dimensions: 6.0 m x 2.8 x 2.5 m – at the highest roof point) belonging to the Experimental Station of Varieties Testing in Karzniczka (φ = 54°29', λ = 17°14', H = 80 m above sea level). The soil used in the experiment came from a farm following the principles of organic farming. It was taken for experiment in July from the arable layer (0-20 cm) from the oat stand. Soil represented a granulometric group- sand, a subgroup - loose sand with an acidic value (pH in KCl = 5.3) and low content of bioavailable components. The soil was dried, sieved through 4 and 2 mm mesh and then mixed with peat (pH 6.5) in a 1:2 ratio (peat: ecological soil). The soil mixture with peat was wetted, mixed and then dried again.

The seeds were sown into plastic tray-fillers with 110 holes (depth 5.5 cm, width 4 cm). 50 seeds were used as a single experimental combination. Due to the requirements of statistical inference, the greenhouse experiment, carried out only in one environment, was planned in a completely random arrangement. A single series of experiments was

adopted in triplicates (Mądry 2007). Tray-fillers with seeds were placed in a greenhouse in a complete randomization system. Before starting the experiment, 400 ml of water was applied to each pot. To compare the effectiveness of water extracts on soybean germination, depending on the method of their application, two combinations of seed treatment were used: a) 24. hour soaking of seeds in extracts, drying in room conditions and then seeding into the soil to a 3 cm depth were performed. The aqueous extracts were prepared according to the procedure used in the 1st stage of testing. b) 24 hour soaking of seeds in distilled water, drying in room conditions, and then seeding into tray-fillers with spot application of the soil extracts (about 3 ml per hole) using a pipette at a depth of 3 cm were performed. Control samples were untreated seeds soaked for 24 hours in distilled water before sowing. Throughout the test, the plants were watered with tap water once a day.

In order to determine the course and pace of soybean growth, for 15 days (assuming the first day after sowing as the first), germinating seedlings were counted. During the experiment, microclimate conditions were controlled. The air temperature in the greenhouse fluctuated between 20°C (night) – 26°C (day), the temperature of the soil was about 22°C and the air humidity was approx. 50%. On the basis of the measurements carried out in the greenhouse test, the indicators indicating the vigor and the seed seeding quality were calculated: Pieper (1) and Maguire (2). Pieper's index (Jakubowski 2015) is defined as the average number of days required to germinate one seed, the Maguire's index determines the relative germination rate (Maguiere 1962):

Pieper's index (W_{Pi}) =

$$\frac{(x1*s1 + x2*s2 + \ldots\ldots + xnsn)}{s1 + s2 + \ldots\ldots + sn)} \tag{1}$$

Maguire's index (W_{Magu}) =

$$\frac{s1}{x1} + \frac{s2}{x2} + \ldots + \frac{sn}{xn} \tag{2}$$

where:
x – subsequent germination days,
s – number of seeds that normally germinated on a given day,
n – last day of the experiment.

2.2. Statistical analysis

Corrected means of the replicates of the examined plant characteristics were calculated based on data from a single series of two-factor experiments carried out in triplicate (for each variety separately, the factors tested are the form of the preparation and the method of application). Means comparisons were performed on two-way ANOVA for each variety separately and multiple comparisons (Tukey test) based on NIR (LSD). For all analyzes, the level of significance was set at $P \leq 0,05$. The analyzes were carried out using the Statistica 13 program.

3. Results and discussion

During the experiment, microclimate conditions were being monitored. These were optimal conditions for soybean germination (Tyagi & Tripathi 1983). Analysis of variance showed a significant impact of the method of application of aqueous extracts on the number of emerged plants of all soybean varieties (Table 1). Seed sowing with simultaneous dosing of preparations had a positive effect on germination and emergence, whereas the reaction of the Merlin cultivar for the technique of application was the largest (average number of plants raised 17.1- "per seeds"; 44.1 – "per soil". The results of tests carried out by other authors confirm the results from the discussed experiment. The germination rate of beet seeds (Czerwińska et al. 2016a) yellow lupine and pea (Czerwińska et al. 2016b) is better when applying extracts- infusions directly to soils than soaking in these preparations prior to seed. The beneficial effect of soil application is due to the fact that active substances contained in extracts may act, among others, as natural biopesticides and show efficacy against pathogens transmitted by seeds and soil (Perello 2013, Sengupta et al. 2008). Vegetable oils are known to be used in biological soil fumigation (Dhingra et al. 2013).

Table 1. Average values of the variety-dependent variables (average number of emergent plants, Pieper's and Maguire's index) tested, the form of the water extract and the method of its application, in relation to the control object (%)

Tabela 1. Średnie wartości testowanych zmiennych zależnych (średnie liczby wzeszłych roślin, współczynnik Piepera i Maguiera) od odmiany, formy ekstraktu wodnego oraz sposobu jego aplikacji z uwzględnieniem relacji wobec obiektu kontrolnego (%)

	Abelina			Augusta			Merlin		
application:	per seeds	per soil	average	per seeds	per soil	average	per seeds	per soil	average
Average number of emerged plants									
1 m	24,66 a	36 b	30,33 A	21,67 ac	32,33 ab	27 A	32,3 c	42,33 ac	37,33 B
2 d	33 ab	38 b	33,17 AB	16,33 c	33 ab	24,66 A	9,33 b	45,67 a	27,5 A
3 i	26,7 a	39,67 b	35,5 B	23,33 abc	37,3 b	30,3 A	9,67 b	44,33 a	27 A
$\bar{X}$	28,11 A	37,88 B		20,44 A	34,22 B		17,1 A	44,1 B	
Average number of emerged plants (% relative to control)									
1 m	-11,93	28,57	100	-32,29	1,04	100	-26,52	-3,79	100
2 d	17,86	35,71		-48,96	3,13		-78,79	3,79	
3 i	-4,76	41,67		-27,08	16,67		-78,03	0,76	

Table 1. cont.
Tabela 1. cd.

application:	Abelina			Augusta			Merlin		
	per seeds	per soil	average	per seeds	per soil	average	per seeds	per soil	average
Maguire's index									
1 m	2,2 a	4,52 c	3,35 B	3,29 cd	4,79 ad	4,04 A	2,85 c	6,21 b	4,63 A
2 d	3,03 a	6,01 b	4,51 A	2,57 bc	5,31a	3,94 A	0,75 a	6,56 b	3,66 B
3 i	2,03 a	5,85 b	3,94 B	1,54 b	5,92a	3,74 A	0,78 a	8,26 d	4,52 A
$\bar{X}$	2,42 A	5,46 B		2,47 A	5,34 B		1,53 A	7,01 B	
Maguire's index (% relative to control)									
1 m	-16,7	71,35	100	7,93	57,07	100	-40,61	29,3	100
2 d	15,02	127,6		-15,57	74,27		-84,33	36,76	
3 i	-22,8	121,9		-49,36	94,23		-83,68	72,04	

Table 1. cont.
Tabela 1. cd.

application:	Abelina			Augusta			Merlin		
	per seeds	per soil	average	per seeds	per soil	average	per seeds	per soil	average
Pieper's index									
1 m	11,7 b	8,49 a	10,1 AB	11,4 b	7,99 a	9,69 A	11,72 b	7,63 a	9,67 A
2 d	11,21 b	7,55 a	9,37 A	11,17 b	7,34 a	9,25 A	13,09 b	7,6 a	9,69 A
3 i	13,37 c	7,68 a	10,52 B	12,19 b	6,95 a	9,57 A	12,98 b	6,4 a	10,34 A
$\bar{X}$	12,08 B	7,9 A		11,58 B	7,42 A		12,6 B	7,20 A	
Pieper's index (% relative to control)									
1 m	6,26	-22,65	100	5,95	-25,6	100	23,67	-19,49	100
2 d	2,07	-31,26		3,87	-31,7		38,13	-19,83	
3 i	21,79	-30,02		13,44	-35,4		37,02	-32,42	

Comment: First column: 1 m – macerate, 2 d – decoction, 3 i – infusion. Average values within the same variety followed by the same letter do not differ significantly ($P \leq 0,05$). Lowercase letters are used to compare averages for combinations: form of the preparation x method of application.

The use of aqueous extracts in a "per seed" combination, i.e. with 24 hour wetting of seeds, generally limited the germination and emergence of all soybean cultivars. Only Abelina variety soaked for 24 hours in the decoction, an improvement in germination and emergence compared to the control was observed. Phytotoxic effect of natural substances may occur due to their high concentration in the prepared extracts. In the studies of Orzeszko-Rywka and Rochalska (2007) the suitability of natural preparations for the seed treatment of sugar beets was tested. It has been shown that soaking seeds in undiluted thyme oil worsens the field's ability to germinate. Undiluted oil of *Zhumeria majdae* leaves - Iranian aromatic and medicinal plant, inhibit germination of seeds and emergence of tomato and wheat seedlings (Soltanipoor et al. 2006). Treatment of seeds of other cultivated varieties, eg: *Allium cepa*, *Lactuca sativa* with an aqueous extract from *Zhumeria majdae* inhibited germination of the tested plants (Soltanipoor et al. 2007). The water extract of *Brassica nigra* leaves inhibits germination and emergency of lentil (Munir et al. 2002), wild oats (Turk & Tawaha 2002) and wild barley (Tawaha & Turk 2003) and the inhibitory effect increases with extract concentration.

The greenhouse test also showed a significant impact of the preparation form on the number of emerged plants of all varieties, however, different forms of preparations improved the value of the discussed variable. Irrespective of the treatment technique, the best results were obtained using infusions – an average of 35.5 plants of the Abelina variety, and macerate – an average of 37.3 plants of the Merlin variety. In contrast, the Augusta variety in which the seed treatment was present was average not statistically significant. However, the interaction of factors was statistically significant. Comparing the experimental results to control group, the greatest improvement in soybean germination was observed using the following forms in soil: macerate (+28.57%), decoction (+35.71), infusion (+41.67) as a seed treatment of Abelina cultivar, infusion (+16,67%) in combination with the Augusta variety, decoction (+ 3.79%) for Merlin variety. Soaking seeds of the Merlin variety in the decoction (-78.79%) and infusion (-78.03%) from *A. hypocastanum* inhibited the germination and emergence of plants to the greatest extent.

The values of germination and emergence indicators, i.e. Maguire and Pieper (Table 1) of all varieties were significantly dependent on the method of application of plant extracts. For the Merlin variety, the form

of the extract used had a significant effect on the relative germination rate. The values of the above indicators determined for the Abelina variety were also significantly dependent on the type of extract used as a treatment. The interaction of factors was also statistically significant. The soil application of extracts based on chestnut flowers improved the dynamics of soybean emergence, i.e. the acceleration of emergence by shortening the average germination time of a single seed was observed. The soil application method improved the vigor of seeds and seedlings. Prolonged and most unbalanced germination and emergence (low Maguire's index, high Pieper's index) was observed in the Merlin cultivar after 24 hours in the soaking of seeds in decoctions and infusions. Abelina and Augusta also germinated slower, but the values of the calculated coefficients were not so unfavorable. The method of application of natural plant preparations can influence the speed and uniformity of the emergence of different species belonging to the same botanical family and varieties. In research by Czerwińska et al. (2016 b) daily treatment of seeds with infusions of *Allium sativum* increased the germination rate and emergence of yellow lupine. For pea seeds, the reverse effect was obtained, similar to the observed in the independent experiment, i.e. the application of the extract from *Verbascum thapsiforme* (flowers) during seeding in the ecological soil determined the higher speed and shortening the time of emergence. The synergism in the action of the active substances contained in the various preparations used together means that the emergence of plants can proceed much faster than for each component separately. Powdered mixture of garlic and basil used for seed treatment by Rochalska & Orzeszko-Rywka (2009) significantly accelerated the field emergence of parsley. It was noted that sugar beet seeds, dinkel wheat, radishes, carrots and parsley reacted differently to active ingredients contained in all applications, but their utilization did not delay field emergence.

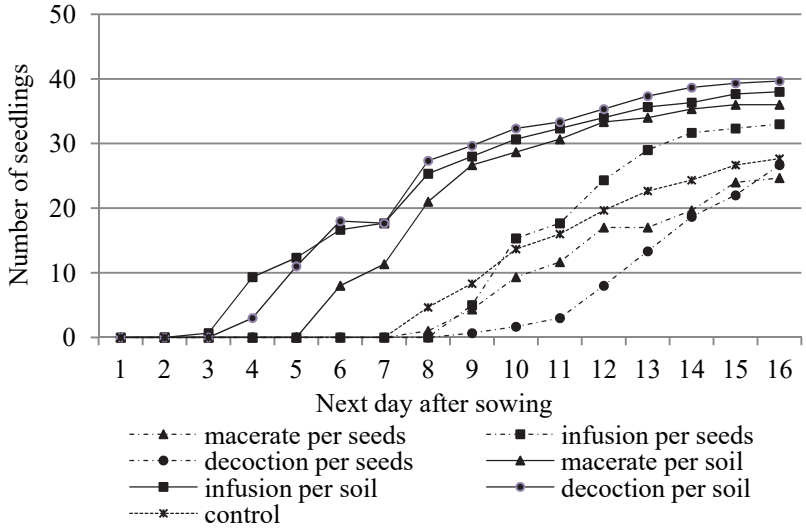

Fig. 1. Number of seeds of the Abelina, which germinated in the following days from sowing

Rys. 1. Liczba nasion odmiany Abelina, które skiełkowały w kolejnych dniach od siewu

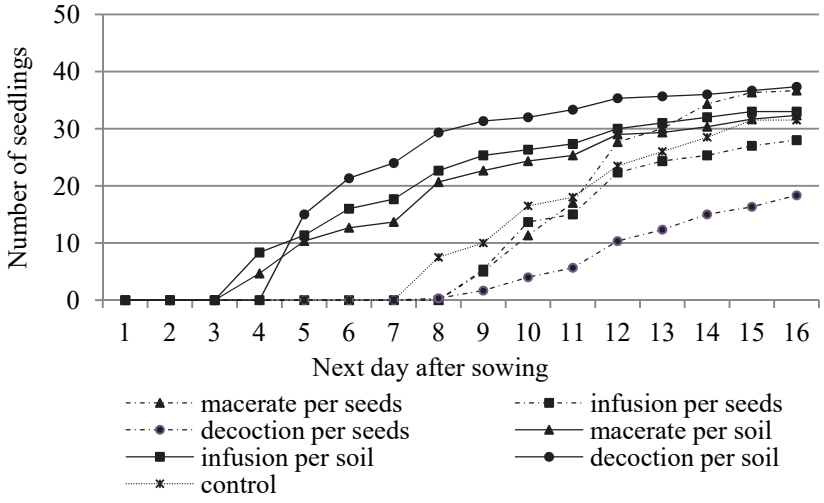

Fig. 2. Number of seeds of the Augusta, which germinated in the following days from sowing

Rys. 2. Liczba nasion odmiany Augusta, które skiełkowały w kolejnych dniach od siewu

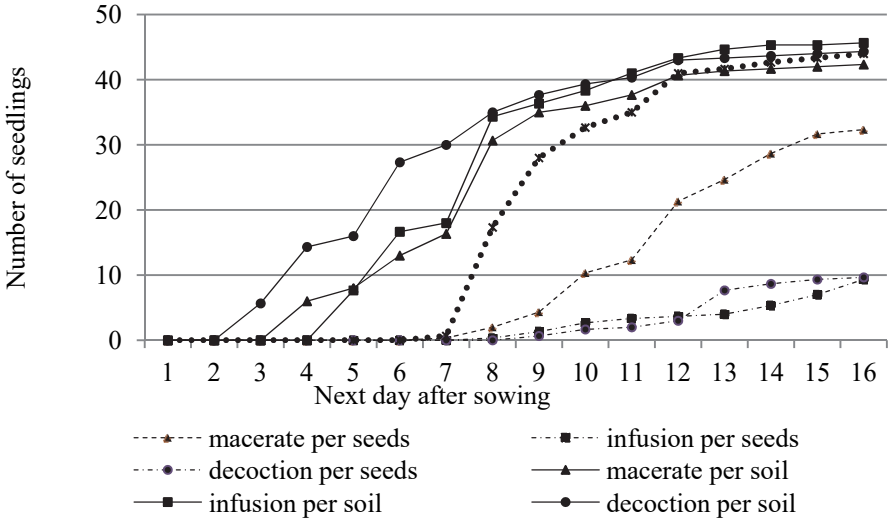

Fig. 3. Number of seeds of the Merlin, which germinated in the following days from sowing
Rys. 3. Liczba nasion odmiany Merlin, które skiełkowały w kolejnych dniach od siewu

Seeds of all varieties of soybean treated during seeding, germinated on the 3rd day of study (Figure 1-3). The first seedlings of control combinations appeared much later – one week after sowing. The seeds soaked in the extracts delayed soybean rising by 1-2 days compared to the control. Seedlings appeared very late and the emergence was the most monotonous. Extending the pre-sowing time of seed soaking in treatment solutions may lead to the weakening of their vigor. In a study conducted by Kaniewska et al. (2012) it was experimentally confirmed that with increasing concentration and prolongation of time of radish seeds storage in aqueous solutions of acetic acid, the vitality of seeds deteriorated significantly. The emerging seedlings were characterized by abnormal growth and their germination energy was low. Seeds soaked for a long time can undergo quicker damage and putrefaction.

The rapid increase in the number of emerging seedlings took place from 4 to 7 (soil application) and from 9 to 11 day of the experiment (seed soaking) (Figure 1-3). The reaction of the Augusta cultivar on seed soaking in macerate was quite unusual. For a long time after sowing, the seeds did not germinate, but after a week the seedlings began to

penetrate intensely through the soil layer. The worst emergence was observed in the Merlin variety after. soaking of seeds in infusion and decoction. The emerging seedlings were low and weak.

4. Conclusions

1. Soil application of water extracts improved the germination effectiveness, quality and vigor of seeds and soybean seedlings. This is evidenced by the favorable development of the calculated coefficients: Pieper and Maguire's.
2. 24 h soaking of seeds in prepared extracts limited germination and soybean emergence. The exception was the Abelina variety, for which the seeds treated with decoction had a positive effect on the number of emerged seedlings and the speed of their emergence.
3. The form of water extract in which the seed treatment is put may have a significant effect on germination and soybean emergence, however, this relationship was not confirmed in all tested varieties.
4. The application of extracts directly to the soil may increase the effectiveness of treatment as a result of decontamination of not only the surface of the seed, but also its surroundings.

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Ocena kiełkowania nasion soi potraktowanych naturalnymi ekstraktami wodnymi w zależności od metody ich stosowania

Streszczenie

Soja (*Glycine max* L.) jest ważnym surowcem do produkcji pasz przemysłowych zarówno w Polsce, jak i w całej Unii Europejskiej. Niestety warunki klimatyczne, dostępne odmiany soi oraz mała podaż preparatów zarejestrowanych w tej uprawie to główna przyczyna niewielkiej produkcji w krajach UE. Przedmiotem niniejszych badań była reakcja trzech odmian soi (*Glycine max* L.): Abelina, Augusta, Merlin na naturalne, wodne zaprawy nasienne sporządzone w formie maceratu, wywaru i naparu na bazie suszu z kwiatów kasztanowca zwyczajnego (*Aesculus hippocastanum* L.). Doświadczenie zostało założone latem w obiekcie szklarniowym należącym do Centralnego Ośrodka Badania Odmian Roślin Uprawnych, Stacji Doświadczalnej Oceny Odmian w Karzniczce ($\varphi = 54^{o}29'$, $\lambda = 17^{o}14'$, H = 80 m n.p.m), zlokalizowanej w województwie pomorskim. W teście szklarniowym przez 16 dni określono średnią liczbę wzeszłych roślin po użyciu wyciągów wodnych. Na podstawie uzyskanych wyników obliczono dwa współczynniki: Piepera i Maguiera. Zastosowano dwie kombinacje aplikacji ekstraktów: dobowe zaprawianie w maceratach, wywarach i naparach, a następnie wysiew do gleby oraz wysiew moczonych w wodzie destylowanej ale niezaprawionych nasion przy jednoczesnym, ręcznym dozowaniu ziołowego wyciągu. Kombinację kontrolną stanowiły nasiona

nie traktowane preparatami, moczone przed dobę w wodzie destylowanej. Wykorzystaną w eksperymencie glebę pobrano w czerwcu z warstwy ornej (0-20 cm) spod uprawy owsa ekologicznego. Przedplonem dla owsa był ekologiczny łubin wąskolistny. Podczas 16 dni eksperymentu kontrolowano warunki temperatury i wilgotności. Wyniki eksperymentu szklarniowego wykazały, że nasiona wybranych odmian soi kiełkowały lepiej i szybciej, gdy zaprawiano je bezpośrednio podczas siewu (aplikacja doglebowa). Stosowanie ekstraktów „na nasiona" ograniczyło kiełkowanie i wschody soi. Dla dwóch testowanych odmian- Abeliny i Merlina, wykazano także istotny wpływ formy zaprawy na uzyskane wyniki w doświadczeniu. Najlepsze wschody zaobserwowano u odmiany Merlin, gdy jej nasiona moczono w wodzie destylowanej a ekstrakty aplikowano punktowo do gleby. Hamowanie kiełkowania i wschodów soi zaobserwowano po dobowym moczeniu nasion w ekstraktach w większości kombinacji doświadczalnych. Konieczne są dalsze badania w celu zidentyfikowania związków bioaktywnych zawartych w wodnych ekstraktach i ocena skuteczności aplikacji doglebowej preparatów w warunkach polowych. Przedstawione badania są niezwykle ważne z punktu widzenia praktyki rolniczej i ochrony środowiska. Poszukiwanie naturalnych metod zaprawiania nasion jest doskonałą okazją dla rolnictwa ekologicznego, gdzie nie dopuszcza się do stosowania zapraw syntetycznych.

Abstract

Soybean (*Glycine max* L.) is one of the most popular crop species in the world, which supplies raw material for the production of commercial fodder in Poland, as well as throughout the European Union. Unfortunately, climatic conditions and available varieties of soybeans and few preparations registered in plant protection are the main reason for low production in EU countries. The subject of the following study is examining the reaction of three varieties of soybeans (*Glycine max* L.): Abelina, Augusta, Merlin on natural seed treatments in the form of macerates, decoctions and infusions of *Aesculus hippocastanum* L. flowers. The experiment was carried out in a greenhouse facility belonging to Research Centre for Cultivar Testing, Experimental Station of Varieties Testing in Karzniczka ($\varphi = 54^{o}29'$, $\lambda = 17^{o}14'$, H = 80 m above sea level) in Pomeranian voivodship. In the greenhouse test, lasting 16 days the average number of sprouted plants were determined after applying water extracts and coefficients: Pieper and Maguire's. Two combinations of extracts were used: 24 h treatment in macerates, decoctions and infusions, followed by soil seeding and distilled water seeding using untreated seeds, while simultaneously dispensing herbal extracts. The control combination were seeds untreated with preparations. The soil used in the experiment was collected in June from the arable layer (0-20 cm) of organic oat field. The

forecrop was organic narrow-leafed lupine. During the 16 days of the experiment, the temperature and humidity conditions were controlled. The results of the greenhouse experiment showed that the seeds of selected soybean varieties sprouted better and faster when treated directly during seeding (soil application). The use of extracts "per seeds" limited emergence of soybean. For the two tested varieties- Abelina and Merlin, a significant effect of the seed treatment form on the obtained results in the experiment was also demonstrated. The best emergence was observed in the Merlin variety, when its seeds soaked in distilled water and the extracts were applied directly to the soil. Further research is needed to identify the bioactive compounds contained in the aqueous extracts and assess the effectiveness of the soil application of the extracts under field conditions. The conducted research is extremely important from the point of view of agricultural practice and environmental protection.

Słowa kluczowe:
Aesculus hippocastanum L., ekstrakty wodne, kiełkowanie, wschody, soja

Keywords:
Aesculus hippocastanum L.*,* natural plant extracts, germination, emergence, soybean