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Reaction of soybean varieties to natural water extracts in different method of their application

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ABSTRACT

The subject of the following study is the reaction of six varieties of soybeans (*Glycine max* L. Merr.): Abelina, Augusta, Merlin, Mavka, Aligaor, Lisabon, on an aqueous decoction of dried *Artemisia absinthium* L. used as wet seed treatment. The experiment was carried out in a greenhouse belonging to the Experimental Station for Variety Testing in Karzniczka. Two combinations of the extract were used: 24h soaking of seeds in the decoction followed by sowing and soaking in distilled water followed by sowing, but untreated seeds with simultaneous dosing of herbal extract. The control group were seeds not treated with preparations. After sixteen days of the test, the average number of plant growth was determined for each experimental combination. The morphological characteristics of the seedlings: length of shoots and roots as well as fresh and dry mass of shoots and roots were evaluated for ten randomly selected plants from each experimental combination. The results of the greenhouse experiment showed different reaction to the application of the decoction in the form of a seed treatment. Soil application of the extract led to the improvement of soybean growth. Seedlings of all varieties, the seeds of which were treated and sown, developed a longer and stronger root system at the expense of a slight reduction in the length and weight of the shoots. The 24h soaking of seeds in the decoction of *Artemisia absinthium* caused the deterioration of emergence and the visible inhibition of the growth and development of soybean seedlings. The inhibition of germination and emergence by the tested extract excludes the possibility of agricultural application. Further research is needed to identify the bioactive compounds contained in the used decoction and to assess the real effectiveness of the soil application of the extracts under field conditions.

***Keywords*:** Soybean, water extracts, *Artemisia absinthium*, seed dressing

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1. INTRODUCTION

The intensive development of agricultural production processing caused by the growing demand for food on a global scale has led to an increase in the use of synthetic production means, including pesticides [1]. Producers take care of their crops and thus try newer and stronger active substances with a broad spectrum of activity. A strong lobby of consumers and ecologists has led to the withdrawal of many active substances from the market, resulting in the reduction of maximum residue levels of plant protection products in unprocessed crop products. For example, in 2013, due to occurrence of scientific reports on the possible harmful effects of seed treatments belonging to neonicotinoids (insecticides) against pollinating insects, the European Union introduced a total ban on their use. From that moment on, the market lacks effective seed treatments in the cultivation of, among others oilseed rape [2]. Searching for alternative solutions for agriculture applications encourages researchers to take a closer look at various natural substances that have antimicrobial properties [3]. It is known that some of them also have indirect effects, i.e. they can stimulate growth and development of plants [4, 5]. There are many natural products available on the market, based on antagonistic microorganisms against disease-causing organisms such as plant extracts or other components rich in biologically active organic compounds. Biopreparations, biotechnical preparations or plant defense stimulators can be used in conventional (integrated) farming [6]. In turn, not all biological preparations available for sale can be used in crops that follow the principles of organic farming. The pool of plant protection products qualified for use in organic farming in Poland, in particular those intended for pest control, is said to be insufficient. Currently, the list includes over 40 products, most of which are preparations for use in horticultural and fruit growing [7]. Due to the lack of preparations dedicated to agricultural crops, the search for new substances of vegetable origin is sought for. It is also worth mentioning that protection of plants against pathogens should be carried out from the very beginning - that is, from sowing/planting. One of the most important yielding factors is good quality and proper seed preparation. The environmental aspect, but also the total ban on the use of chemical treatments in organic farming, favors greater interest in substances less harmful to the environment, which could be used as seed treatments.

The aim of the following study is to determine the effect of natural decoction made on the basis of *Artemisia absinthium* L. on the ability of emergence and initial growth of seedlings of six varieties of soybeans depending on their application method.

2. MATERIALS AND METHODS

A two-factor greenhouse experiment was carried out in a facility of Experimental Station for Variety Testing in Karzniczka ($\varphi = 54°29'$, $\lambda = 17°14'$, H = 80 m a. s. l.) in August. The experiment used a decoction of *Artemisia absinthium* made according to the procedure proposed by Sas- Piotrowska and Piotrowski [8]. The decoction was used as a natural wet seed treatment. The first factor of the experiment was 6 varieties of soybeans: Abelina, Aligator, Augusta, Lisabon, Mavka, Merlin. The method of application of decoctions, the second factor of experience, was present in the following combinations: 24-hour soaking of seeds in the decoction, drying of seeds, soil sowing; 24-hour soaking of seeds in distilled water, drying of seeds, soul sowing with simultaneous application of the preparation (3 ml of

decoction per 1 seed). The seeds were soaked for 24 hours in distilled water. Seeds were sown into plastic tray-fillers with 110 pots. Each pot contained two different experimental combinations. Each experimental combination (50 seeds) were performed in triplicate. The soil used in the experiment was a mixture of sifted soil from the arable layer from the ecological oat stand and peat of pH 6,5 (1: 2). Before sowing, 400 ml of tap water was added into each tray-filler pot in order to ensure proper humidity. The greenhouse test was carried out for 16 days from the day of sowing. Plants growing in natural light were watered once a day with tap water. On the last day of the experiment, all the seedlings were counted. From each experimental combination, 10 plants were randomly selected and carefully washed and dried. The length of roots and shoots was measured using an electronic caliper (accuracy +/- 0.01 mm). Next, the plants were divided into 2 parts: above and underground. Roots and shoots were weighed on a laboratory electronic scale (accuracy +/- 0.01 g). Fresh plant material was subjected to drying for 8h at 65 °C in a SLW 240 STD laboratory dryer. The obtained dried plant was weighed.

To assess the impact of individual experimental factors on the results obtained in the greenhouse test, a two-factor analysis of variance was used at the 95% significance level. In addition, multiple comparisons were made with the use of Tuckey test and homogeneous groups. Analysis of the main components (PCA) allowed to evaluate the relationship between all tested variant features and to indicate the similarity between varieties. The results of the analysis are presented in the form of a graph of the first and second component (PC1 and PC2).

3. RESULTS AND DISCUSSION

3. 1. The effect of various seed treatments based on *Artemisia absinthium* decoction on the number of emerged soybeans.

The carried out statistical test showed a significant impact of both experimental factors, i.e. the soybean variety and the method of application of a natural preparation on the average number of emerged plants in the greenhouse test. The varieties reacted differently to the application of *A. absinthium* decoction as the seed treatment. Irrespective of the application technique used, the variant with the best emergence was the Merlin variety - an average of 35.66 plants, the worst was Mavka - an average of 22.33 plants. In two combinations with the use of decoction the average number of seedlings increased, comparing to the combination without seed treatment. This dependence was only not confirmed by Augusta variety. In research by Kolasinska [9] on the influence of natural treatment methods on the germination and vigor of spring variety of cereals coming from organic farming, a differentiated reaction was observed in oat, wheat and barley varieties to natural seed treatments (garlic water suspension, milk powder, hot water, 10% solution of baking soda). Generally, none of the used treatments have improved the germination yield, for all the cereal species tested, the best way of treatment was by aqueous garlic suspension. In an experiment led by Bhaskara Reddy et al. [10] treatment of caryopses of two cultivars of spring wheat: Norseman and Maks using chitosan solution at concentrations of 2 to 4 mg/ml resulted in a significant improvement in germination and the vigor of seedlings in comparison to untreated seeds. The application of chitosan stimulated the synthesis of phenolic acids and precursors of lignins in germplasm

that have antimicrobial properties. It was shown that the Maks variety sprouted a little poorer and the seed vigor was lower compared to the Norseman cultivar.

In the Maks variety, lower induction of phenolic acids and lignin was observed, which resulted in lower resistance of young seedlings to pathogens. Genotype, environment and method of seed treatment have an impact on soy productivity and seed vigor. Although the genetic factor plays a key role in determining the quality of soybeans, the environmental conditions in which the seeds arise and emerge as well as storage conditions determine the longevity and vitality of seeds [11]. The organic treatment application method was the factor with confirmed statistical significance. The greenhouse test showed that 24h soaking of the majority of soybeans with the *A. absinthium* decoction significantly reduced the germination and emergence of seedlings. The strongest reduction in the number of germinating seeds was observed in the following varieties: Aligator, Augusta, and Lisabon. The exception was Abelina cultivar, for which the above method of treatment resulted in visibly better plant emergence. Seeds that were applied natural treatments in the form of extracts or essential oils can lower the sowing value of seeds and seedlings. Especially when they are used in relatively high concentrations, e.g. oil from *Eucalyptus citriodora* [12]; *Cinnamomum zeylanicum*, [13] or without dilution - thyme oil [14]. On the other hand, Ibiam et al. [15] argue that long-term soaking of seeds in preparations, excluding the stage of soaking seeds in water, is more effective, because the contained active substances better penetrate the seeds and stop the metabolic processes more effectively. Some of them act against fungi, thus preventing their development. The inhibitory effect of natural plant extracts is tested mainly on the possibility of their use as natural herbicides, limiting the germination of weeds [16, 17]. Some studies have proved the positive effect of aqueous extracts on germination and plant emergence. The infusion of *Allium sativum* bulbs and *Verbascum thapsiforme* flowers stimulates the germination of yellow lupine and pea [18]. As in the independent field study, on the emergence of legumes, it was better to apply the extracts directly to the soil, not on seeds.

Table 1. The number of emerged plants and morphological characteristics of soybean seedlings depending on the method of application of the decoction from *A. absinthium.*

Application	**Soybean variety**						
	Abelina	**Aligator**	**Augusta**	**Mavka**	**Merlin**	**Lisabon**	**average**
Average number of emerged plants							
per seeds	34,66 cd	26,33 abc	16,33 a	21,66 ab	35,33 cd	21,66 ab	26 A
per soil	16,33 a	38,33 d	32,33 bcd	23 ab	36 cd	32,66 bcd	29,77 B
average	25,5 AB	32,33 BC	24,33 A	22,33 A	35,66 C	27,17 AB	
control	**24,2**	**31**	**30**	**22**	**35,6**	**24**	**27,8**
Average shoots lenght* [cm]							
per seeds	8,13 a	7,13 a	5,4 a	7,91 a	6,97 a	8,22 a	7,29 A
per soil	10,5 a	8,37 a	10,37 a	8,21 a	9,77 a	9,97 a	9,53 B
average	9,32 A	7,75 A	7,88 A	8,07 A	8,37 A	9,09 A	
control	**10,78**	**8,56**	**10,98**	**8,22**	**12,36**	**10,1**	**10,17**

Average roots lengt* [cm]							
per seeds	15,04 ab	12,83 ab	12,26 ab	11,98 ab	13,50 ab	10,91 b	12,75 A
per soil	16,85 ac	13,82 ab	15,21 ab	14,18 ab	20,8 c	16,36 ac	16,2 B
average	15,95 AB	13,32 A	13,74 A	13,08 A	17,14 B	13,63 A	
control	**16**	**12,91**	**15,1**	**12,6**	**19,81**	**11,14**	**14,59**
Shoots fresh mass** [g]							
per seeds	6,99 ab	7,40 ab	6,43 ab	7,77 abc	6,28 ab	5,41 b	6,73 A
per soil	8,08 abc	8,77 acd	11,82 d	10,99 cd	10,66 acd	7,76 abc	9,68 B
average	7,54 AB	8,13 AB	9,13 A	9,39 A	8,48 AB	6,57 B	
control	**8,2**	**9,01**	**11,96**	**11,01**	**10,98**	**7,99**	**9,86**
Roots fresh mass** [g]							
per seeds	5,52 ab	5,77 ab	7,55 ac	5,09 ab	6,11 ab	4,10 b	5,68 A
per soil	6,01 ab	6,95 abc	9,3 c	5,91 ab	7,24 a	5,32 ab	6,79 B
average	5,77 AB	6,36 AB	8,42 C	5,5 AB	6,68 BC	4,63 A	
control	**5,65**	**5,99**	**8,35**	**5,45**	**6,59**	**4,45**	**6,08**
Shoots dry mass** [g]							
per seeds	1,26 a	0,9 a	1,41 a	1,48 a	1,32 a	0,84 a	1,2 A
per soil	1,39 a	1,29 a	2,38 b	2,2 b	1,26 a	1,31 a	1,64 B
average	1,33 A	1,1 A	1,9 B	1,85 B	1,29 A	1,08 A	
control	**1,42**	**1,41**	**2,54**	**2,6**	**1,35**	**1,36**	**1,78**
Roots dry mass** [g]							
per seeds	1,03 ab	0,55 a	0,72 ab	0,6 a	0,64 a	0,55 a	0,68 A
per soil	1,1 ab	0,9 ab	1,24 b	0,64 a	1,02 ab	0,59 a	0,91 B
average	1,04 B	0,72 AB	0,98 B	0,62 A	0,83 AB	0,57 A	
control	**1,03**	**0,68**	**1,1**	**0,62**	**0,78**	**0,57**	**0,8**

Comment: 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: variety x method of application.
* values per one plant
****** values for 10 randomly selected plants

3. 2. The effect of various seed treatments based on *Artemisia absinthium* decoction on morphological characteristics of varieties

The length of shoots and the roots of the evaluated soy varieties were significantly differentiated by the treatment application method. Both the roots and shoots of seedlings coming from soaking of seeds in a 24h decoction were significantly shorter than the roots and shoots of seedlings, which were treated directly during sowing and plants from control group. The seedlings were weaker and more fragile, which is confirmed by the low values of fresh and dry shoots and roots. Allelopathic and antimicrobial properties of preparations based on plants of the genus *Artemisia* have been well understood [19, 20]. Extracts based on *Artemisia annua* inhibit germination of barley seeds as well as growth and development of seedlings [21]. The use of *Artemisia absinthium* decoction into ground caused visible inhibition of the length and reduction of the mass of shoots of all soy varieties in favor of the length and weight of the root system. In comparison to the control object, the average root length of the

tested soybean varieties, the seeds of which were treated during sowing, increased by 1.6 cm. The largest increase in root length was observed in Lisabon (average + 5.22 cm) and Mavka (+ 1.58 cm). The roots of these varieties were thicker, more branched, and confirm the growth of their fresh and dry matter. Plants with a deep and stable root system show high adaptive properties to edaphic limitations and stress conditions [22].

It is worth emphasizing that in dry environments or with high soil salinity, plants can reduce the length and mass of the root system and shoots [23]. Often the only guarantee of survival of a plant is the development of adequate root system [24, 25]. The discussed range can be found in literature research results, for example, on the application of plant phytohormones produced by bacteria that cause an improvement in plant root growth under salinity conditions [26] or water stress [27].

Our independent research indicates the effectiveness of other preparations, the success of which depends on the method of application. The soil application of *Artemisia absinthium* decoction, including seed sowing having a positive effect on the development of the root system, may be recommended for crops at risk of stressful conditions.

The genetic factor significantly differentiated the cultivars in terms of the majority of morphological features of seedlings. The only parameter for which no statistically significant differences were found was the length of the shoots.

3. 3. PCA analysis

Analysis of the main components for the control group combination (Figure 1), the combination of "per seeds" (Figure 2) and "per soil" (Figure 3) showed that the first two PC1 and PC2 main components in each case explain a large percentage of total volatility.

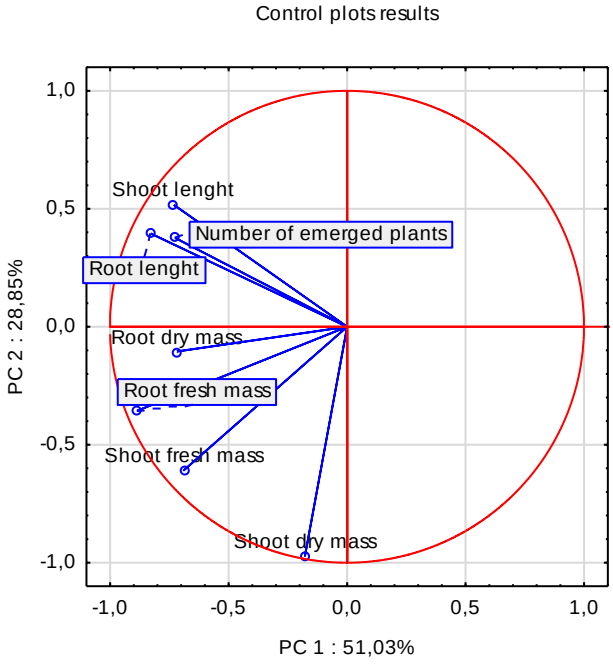

Figure 1. Principal component analysis (PCA) between all traits across cultivars for control combination.

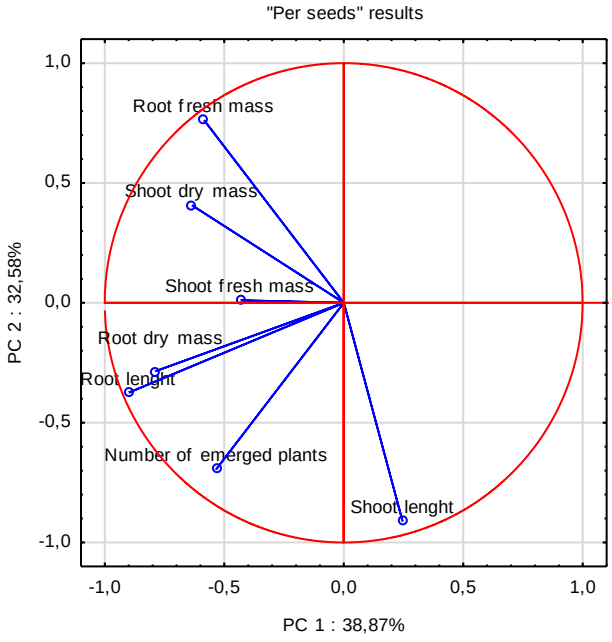

Figure 2. Principal component analysis (PCA) between all traits across cultivars for "per seeds" combination.

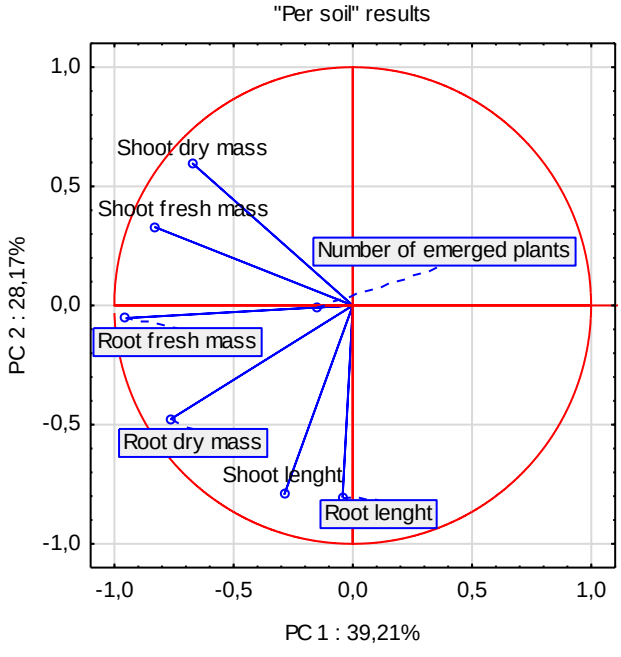

Figure 3. Principal component analysis (PCA) between all traits across cultivars for "per soil" combination.

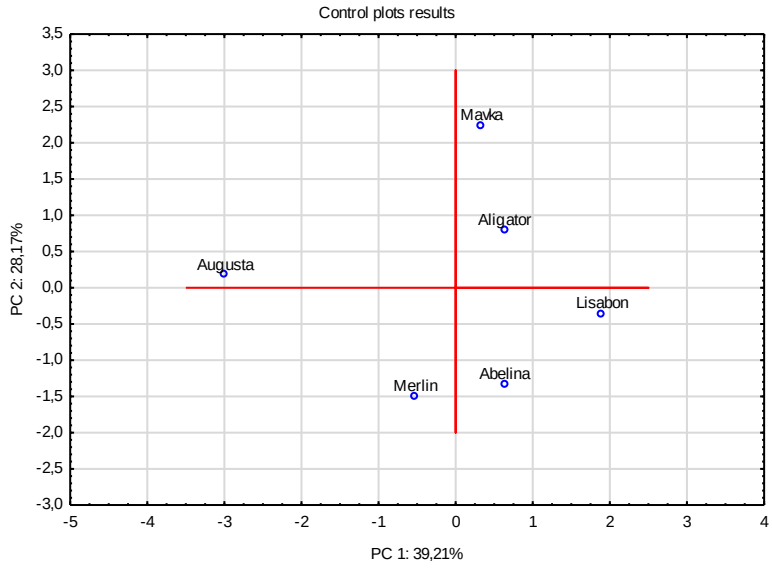

Figure 4. Principal component analysis (PCA) – multi-feature characterization of varieties for control combination.

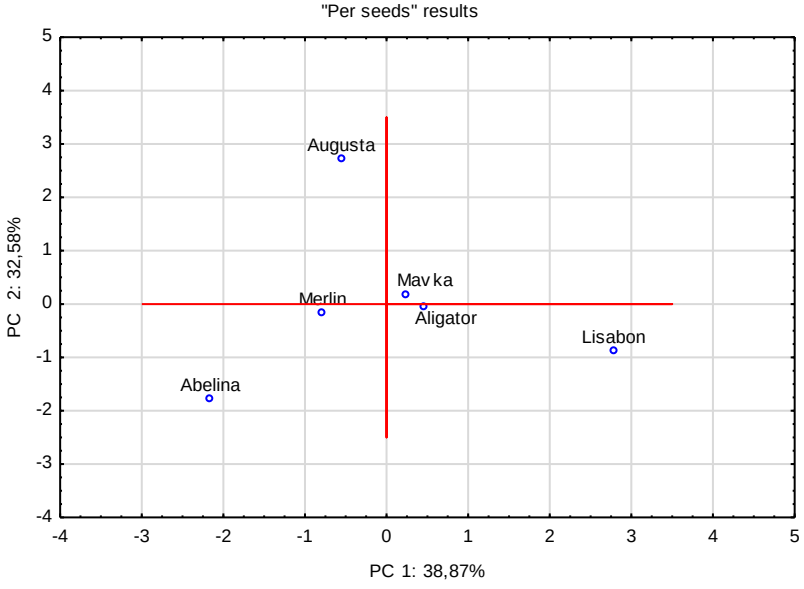

Figure 5. Principal component analysis (PCA) – multi-feature characterization of varieties for "per seeds" combination.

Analysis of the variable components for the control group combination showed a very close correlation between root length and the number of emerged plants. At the same time, these two features correlated with the length of the shoots, but to a lesser extent. Other significant positive correlations are fresh and dry matter of roots and shoots. In "per seeds"

combinations, a positive correlation was also found between the root length and the number of emerged plants, however not as strong as in the controls.

The longer the shoots were forming the plant, the stronger the reduction in the fresh weight of the roots. Positive, strong correlation between the length of shoots and roots was characteristic for "per soil" combination. A strong positive correlation was observed for the number of newly planted plants and fresh root matter.

Figures 2 and 3 show that the contribution of the variable "number of emerged plants" to the construction of the main components for the "per seeds" and "per soil" combination was small. The reaction of soy varieties to the decoction of *Artemisia absinthium* fluctuated and varied depending on the extract technique applied. The variations in close proximity in diagrams 4-6 are similar in all the parameters analyzed (Table 1).

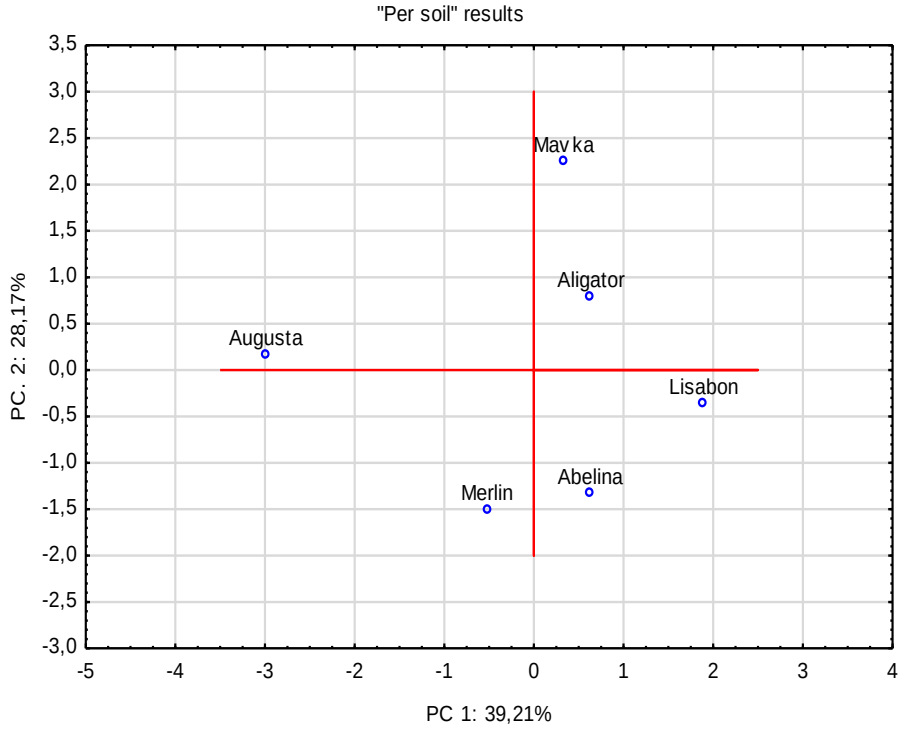

Figure 6. Principal component analysis (PCA) – multi-feature characterization of varieties for “per soil” combination.

4. CONCLUSIONS

The soil application of *Artemisia absinthium* decoction contributed to the improvement of germination and emergence of seedlings on the majority of tested soybean varieties. The exception was the Abelina cultivar, for which the "per seeds" treatment more effectively improved germination and emergence. All varieties whose seeds were sown together with the treatment formed a more developed and extended root system compared to the other two experimental combinations.

The application of a decoction based on *A. absinthium* resulted in a slight reduction in the length, fresh and dry matter of the plant shoots. Applying the extracts directly to the soil can increase the effectiveness of the treatment as a result of decontamination of both the seed area as well as the surroundings. Daily soaking of the seeds caused a slight reduction in the number of germinating seeds and emerging seedlings, limiting the growth and development of emerging plants. Seedlings of all varieties, the seeds of which were treated in the above manner, were shorter, moreover the above- and underground parts were less developed. Due to the inhibitory effect of the *A. absinthium* extract it is not recommended to be applied directly on seeds in the form of a wet seed treatment.

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