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ASSESSMENT OF THE POTENTIAL FOR THE IMPROVEMENT IN GERMINATION CAPACITY OF LEGUMINOUS PLANTS BY MEANS OF PLANT EXTRACTS

Summary

The experiment presented in this article contributed to the assessment of the potential applicability of plant extracts for in-soil treatment of leguminous plant seeds. Germination capacity of yellow lupine (Lupinus luteus L.*) 'Taper' variety and field pea (Pisum sativum* L.*) 'Milwa' variety seeds was assessed after pre-treatment of the seeds with aqueous extracts of green plants selected in earlier in vivo laboratory tests. The extracts consisted of garlic (Allium sativum) bulb infusion for the treatment of yellow lupine seeds and dense-flowered mullein (Verbascum thapsiforme) flowers infusion for the treatment of field pea. Direct-soil germination of seeds was assessed in three combinations. The first combination used as the control combination consisted of non-treated seeds being sown into the soil; the second combination consisted of seeds pre-treated with plant extracts before being sown into the soil, while the third combination consisted of non-treated seeds being sown into the soil locally pre-treated with plant extracts. The experiments were carried out for two soils differing in physical characteristics. Direct application of plant extracts into the soil showed to have better impact on germination when compared to the pre-treatment of seeds, with the response observed in field pea being higher than that in yellow lupine. At the same time, probable factors that inhibited germination were also identified.*
Key words*: bean seeds, water extracts, crop emergence*

OCENA MOŻLIWOŚCI POPRAWY KIEŁKOWANIA NASION ROŚLIN BOBOWATYCH POPRZEZ ZASTOSOWANIE WYCIĄGÓW ROŚLINNYCH

Streszczenie

W pracy wykorzystano wyniki zdolności kiełkowania nasion łubinu żółtego (Lupinus luteus L.*) odmiany 'Taper' i grochu siewnego (Pisum sativum* L.*) odmiany 'Milwa', po zaprawieniu ich wyciągami wodnymi z roślin zielonych, wybranych we wcześniejszych badaniach laboratoryjnych in vivo. Stymulujące działanie na kiełkowanie nasion wybranych roślin w przypadku łubinu żółtego, wykazał napar z cebul Allium sativum (czosnek zwyczajny), a grochu siewnego napar z kwiatów Verbascum thapsiforme (dziewanna wielokwiatowa). W artykule wymienione wyciągi wodne zastosowano w do oceny wschodów roślin bobwatych w glebie. Próbami badanymi były nasiona zaprawiane w/w wymienionymi naparami i w tej formie wysiewane oraz nasiona niezaprawiane wysiewane w miejscu wcześniejszej aplikacji punktowej naparów. Próbą kontrolną były nasiona niezaprawione wysiane do gleby bez aplikacji wyciągów. Badania przeprowadzono w dwóch różnych rodzajach gleby. Pochodziły one z upraw ekologicznych oraz konwencjonalnych. Stwierdzono, że w warunkach glebowych na wschody roślin bobowatych lepiej działało aplikowanie wyciągów do gleby, a nie na nasiona, przy czym lepiej zareagowały nasiona grochu siewnego. Gleba z ekologicznego systemu gospodarowania okazała istotnie lepsza dla wschodów wybranych roślin.*
Słowa kluczowe*: nasiona bobowate, wyciągi wodne, wschody roślin*

1. Introduction

In Poland, the seeds of leguminous plants are used mostly for animal feed production (66%), with lupine predominantly yellow lupine, pea and broad bean being the most important crops. The main advantage of these seeds consists in the high content of proteins required for the production of materials used in swine and poultry farming [5]. Due to the nodule bacteria being present within their rhizospheres, leguminous plants are also commonly used as forerunner crops for subsequent cultivation of cereal plants. As the method is broadly practiced, some agricultural product suppliers offer leguminous plant seeds coated with *Rhizobium* bacterial strains. This results in microorganisms having a beneficial impact on the environment of the seedlings being introduced into soil [7]. The harvest yields of the leguminous plant seeds may vary. The plants are characterized by high requirements, being particularly sensitive to temperature fluctuations as well as to the humidity and overall quality of soil. Pea and broad bean require pH-neutral solid soils while the optimum yellow lupine crops are gathered from light, sandy and acidic soils. Also important is the temperature of germination, with pea and broad beans being particularly sensitive to low temperatures in the germination phase [5].

Considering the above and aiming at achieving the highest possible seed harvest yield, one should attempt and seek for methods to improve the yield of germination that impacts the future harvest. Thus, the studies were aimed at demonstration of the possible use of the extracts of plants for the improvement of conditions affecting germination capability and seedling growth.

2. Materials and methods

The study uses non-treated seeds: crop (from Headquarters Seed): yellow lupine (*Lupinus luteus* L.) of 'Taper' variety, field pea (*Pisum sativum* L.) of 'Milwa' variety were used for the experiments.

Used in *in vitro* investigations plant preparations were made in the form of aqueous extracts, as brews according to the recipe given by Sas-Piotrowska et al. [4, 12]. Brew (hot method) – 5 g of dried plant poured of 250 ml of boiling water and left covered for 30 minutes, after cooling the extract was filtered. Extracts were sieved through a sieve lined with gauze to glass containers, and after cooling, used for the investigations.

The soil were taken from the two farming systems: traditional and ecological. Soils collected from the humus level with a thickness of 0.20 cm. Next the samples were blended, dried, comminuted and sieved using sieve with a mesh of 2 mm.

The tests were carried out using a light soil with moisture content of 85%, pH of 6.0, phosphorus content of 0.0026 g/100g of soil, potassium content of 0.0013 g/100g of soil and magnesium content of 0.0096 g/100g of soil free of chemical fertilizers or herbicides (ecological field). From the traditional system of farming authors used loamy sand brown earth soil with winter wheat as the forerunner crop and oats and broad beans as the soil-mulching catch crop. Next Roundup were used, followed by cultivator tillage. Chemical composition of soil with moisture content of 85% was as follows, pH of 5.72_{KCl}, phosphorus content of 0.0112 g/100g of soil, potassium content of 0.0180 g/100g of soil and magnesium content of 0.0039 g/100g.

The test was carried out in laboratory conditions. Flower-pot experiments were carried out in 40 plastic Seed bed containers 50 mm in diameter and 50 mm in depth. Forty seeds of selected crops were planted into each soil-filled container at the depth of 1.5 cm. Containers with seeds were placed in a chamber containing 400 mL of water that was uniformly soaked up into each flower pot to achieve uniform soil moisture of 80%. in each flower pot. The ambient temperature of 18°C was maintained throughout the experiment.

Two combinations were used to compare the efficacy of water infusions on the seedlings as follows:

- the sowing material was prepared by soaking the plants for 24 hours in aqueous extracts; subsequently, the seeds were dried in air and sown into the two types of soil;
- non-treated seeds were sown into the two types of soil at the locations of topical application of the respective extract.

The experiment was conducted in quadruplicate for each combination, with non-treated seeds sown into the non-treated soil used as the control group. The germinating plants were counted each day. Experiment was carried out 15 days.

Plant extracts chosen for seed treatment and direct soil applications were those demonstrated to have the best seed germination-stimulating effects, i.e.

- garlic (*Allium sativum*) bulb infusion for yellow lupine,
- dense-flowered mullein (*Verbascum thapsiforme*) flowers infusion for field pea [4].

Between post-sowing Day 1 and Day 15, the seedlings were systematically counted with identification of newly emerging seedlings performed so as to determine the mean germination capacity and the course of plant germination. The temporal profile of germination was used for calculation of the mean time for germination of a single plant and expressed by Pieper's (1) index (the day of appearance of the first seedlings taken as Day 1) and the Maguire's rate of germination (2) [3].

Rate of Pieper's = Σ(dn·an)/Σan (1)

where:

dn –means the day of the east plant

an – the number of seeds germinated at given intervals of time

Rate of Maguire's = Σ(ni/ti) (2)

n_i – the number of seeds germinated at given intervals of time

t_i – time seed germination

Statistical analyses: the results were processed statistically by the analysis of variance with single class (P = 95%). If significant differences were detected, the lowest significant difference $LSD_{0.05}$ was calculated (0.05=Tukey's confidence interval value).

3. Results and discussions

Comparison of the reactions of leguminous plants to aqueous treatment solutions revealed that infusions of garlic (*Allium sativum*) bulbs and dense-flowered mullein (*Verbascum thapsiforme*) flowers had a positive impact on the viability and health of the seeds of yellow lupine and field pea, respectively (Table 1). The results from table 1 were derived from previously conducted investigations on energy and capacity of germination and microbial contamination. On the basis of these results, the choice of plants and extract preparation method were made [4].

As shown by data included in Tables 2-3, individual species of leguminous plants presented with different responses to the plant extracts used for seed and soil treatment. Best effects were observed for direct soil application of aqueous extracts prior to seed sowing. Pre-sowing application of plant extracts into ecologically cultivated soil led to increased germination capacity of yellow lupine and field pea, the respective values being +14.72% and +28.89%. In case of field pea, mean germination time as expressed by Pieper's index was lower thn that in the control group for both methods for the management of germination environment. The most sensitive indicator of the germination process, i.e. the Maguire's rate, was significantly increased (as compared to controls) for both pre-sowing treatment of pea seeds and direct soil application of dense-flowered mullein infusion.

Table 1. The aqueous extracts of the best impact on germination of seeds of Fabaceae (% relative to control)

Tab. 1. Wyciągi wodne o najlepszym oddziaływania na kiełkowanie nasion roślin bobowatych (% w stosunku do kontroli)

Tested seeds	Plant species	Form extract	E1 %	Z1 %	Z5 %
Yellow lupine	Bulbs of *Allium sativum*	brew	+11.46	+10.49	-42.86
Field pea	Flowers of *Verbascum thapsiforme*	brew	+4.90	+7.69	-78.95

E1 – *germination energy*, Z1 – *capacity of germination*, Z5 – *microbial contamination*

Source: own work / Źródło: opracowanie własne

Table 2. The ability, uniformity and speed of emergence of Fabaceae plants according to the method of sowing seeds in the soil from the ecological farming
Tab. 2. Zdolność, równomierność i szybkość wschodów roślin bobowatych w zależności od sposobu siewu nasion do gleby z upraw ekologicznych

	Yellow lupin			Pea seed		
	seed	soil	control	seed	soil	control
The ability of emergence %	69	78	68	84	98	76
$LSD_{0.05}$/ $NIR_{0.05}$	7.00			7.46		
The ability of emergence % relative to control	seed		soil	sedd		soil
	+1.47%		+14.72%	+10.53%		+28.95%
Rate of Pieper's	4.59	5.82	4.46	4.74	4.86	4.90
$NIR_{0.05}$ – $LSD_{0.05}$	1.06			0.56		
Rate of Pieper's uniformity of emergence [days]/	2.67	2.82	1.77	2.74	2.89	1.90
$LSD_{0.05}$ - $NIR_{0.05}$	0-74			0.97		
Rate of Maguiere's	20.80	17.95	19.42	23.83	27.21	20.08
$LSD_{0.05}$ - $NIR_{0.05}$	3.49			6.56		

Source: own work / Źródło: opracowanie własne

Table 3. The ability, uniformity and speed of emergence of Fabaceae plants according to the method of sowing seeds in t the soil 21 days after application of the formulation with glyphosate
Tab. 3. Zdolność, równomierność i szybkość wschodów roślin bobowatych w zależności od sposobu siewu nasion do gleby po 21 dniach od zastosowania preparatu z glifosatem

	Yellow lupin			Pea seed		
	seed	soil	control	seed	soil	control
The ability of emergence %	9	3	13	13	22	21
$LSD_{0.05}$ - $NIR_{0.05}$	5.36			6,75		
The ability of emergence % relative to control	seed		soil	sedd		soil
	-30.77		- 76.92	-38.10		+ 4.76
Rate of Pieper's	6.89	9.0	5.54	7.61	6.46	5.85
$NIR_{0.05}$ – $LSD_{0.05}$	1.73			1.34		
Rate of Pieper's uniformity of emergence [days]/	3.89	3.00	2.54	3.62	3.46	2.86
$LSD_{0.05}$ - $NIR_{0.05}$	1.23			1.13		
Rate of Maguiere's	1.88	0.39	3.17	2.24	4.97	2.86
$LSD_{0.05}$ - $NIR_{0.05}$	1.67			1.46		

Source: own work / Źródło: opracowanie własne

Positive effects of plants on germination capacities were also observed by Rochalska and Orzeszko-Rywka [11] who used powdered garlic, basil and thyme for the treatment of seeds. Best effects were observed by the authors for spelt seeds being pre-treated with garlic (91% increase in field germination) and parsley seeds being pre-treated with a mixture of garlic and basil – 207% increase in field germination as compared to non-treated seeds. However, numerous studies suggest that the form of the plant extract as well as the application method are important for the obtained effects.

The results obtained by Orzeszko-Rywka, Rochalska, and Chamczyńska [8] suggest that the treatment of seeds with natural plant oils led to different outcomes for different plant species. Only the treatment with cinnamon oil led to no significant reduction in field germination and harvest yields of all tested plants. Significantly different germination capacities were observed for field cultivation of lupine and pea seeds in conventionally cultivated soil with glyphosate fertilizer had been used 21 days prior to the experiment. In the case of yellow lupine, both seed treatment and direct soil application of garlic bulb infusion reduced the germination capacity in the range of 30 to nearly 77% compared to the control group. Statistically significant differences in germination capabilities were observed for different soil environments (from the statistical analysis $LSD_{0.05\ soil\ type} = 4.28$). The problem of non-uniform and poor germination (high Pieper's index and very low Maguire's rate values) of lupine was therefore due mainly to the origin of the soil used in the tests.

Analogous situation was observed in the case of field pea. Only in one case an increase in percentage germination rate was observed, namely following direct soil application of the plant extract (ca. 5% increase compared to the control seeds). The differences in the numbers of seedlings were significant both with respect to the soil used for the sowing (from the statistical analysis $LSD_{0.05\ soil\ type} = 4.86$), as well as application of brews method (from the statistical analysis $LSD_{0.05\ treatment\ mode} = 6.06$).

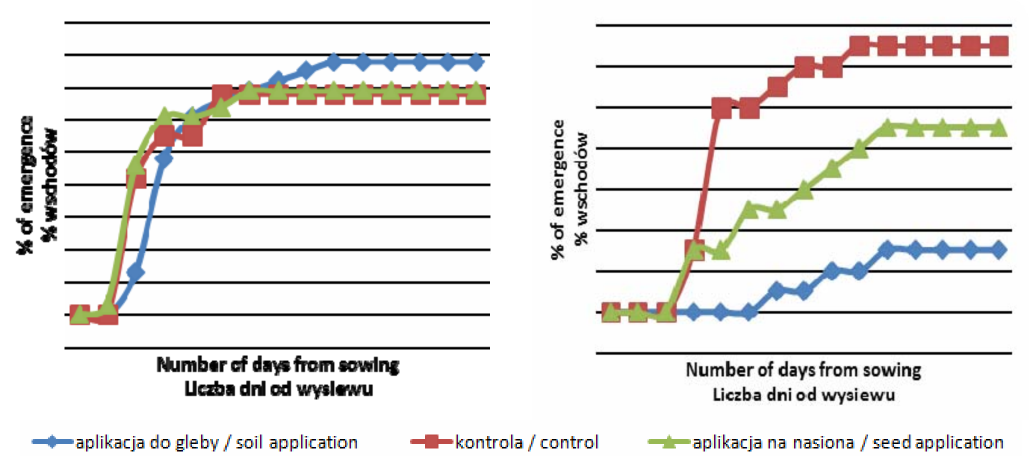
Our experiments showed that the germination capacity, Pieper's index, germination uniformity and Maguire's rates were significantly better in two situations: Firstly, following in-soil application of garlic bulb or dense-flowered mullein flower infusion Secondly, following the sowing of seeds in ecologically cultivated soil in which no chemical plant protection agents had been used. The trends were confirmed for the seeds of both species of leguminous plants. According to Martini, et. al [6] such large discrepancies in the numbers of seedlings, mean germination times or germination uniformity are due to the fact that products used to eliminate unwanted weeds contain the active substance, glyphosate, along with numerous additives enhancing its herbicidal effect. Unfortunately, the additives are often much more toxic than glyphosate itself.

Germination of yellow lupine (Fig. 1a) in the ecologically cultivated soil starter as early as on the second day after sowing in case of plant extract-treated seeds and on the third day after sowing in case of the control seeds as well as in the case of direct soil application of garlic bulb extract. During the first days of the germination period (days 5, 6, and 7 after sowing), the mean percentages of seedlings obtained from non-treated seeds, pre-treated seeds, and seeds planted in pre-treated soil were at similar levels. However, after 15 days of the experiment, it was observed that the direct soil application of plant extract resulted in mean germination capacity of 78%. Overall germination capacity in the remaining combinations (control seeds and pre-treated seeds) was comparable and amounted to 68-69%.

Germination of yellow lupine in the other type of soil (Fig. 1b) started later compared to the case described above. In case of control seeds and plant extract-treated seeds, germination started on day 4 after the sowing. In the case of seeds planted into plant extract-treated soil, germination was observed as late as after 7 days. Unfortunately, differences in the dynamics of germination were also observed between individual combinations. When lupine seeds were sown into the soil that had been pre-treated with glyphosate-containing herbicide 3 weeks earlier, germination capacity compared to that observed in ecologically cultivated soil was reduced by a factor of 26 in the case of combination involving in-soil application of the plant extract, a factor of 5 in the case of control seeds and a factor of 7 in the case of pre-treated seeds. Similar observations were made by Adomas et al. [1, 2] and Piorowicz-Cieślak and Adomas [10] who concluded that soil active herbicides may permeate into surface waters and migrate into the plants from soil, water, and air. Thus, chemical agents may impact physiological processes occurring in plants and have effect on the harvest yields.

Germination of field pea in the soil free of chemical herbicidal agents (Fig. 2a) started on the second day after sowing for the seeds and the soil pre-treated with dense-flowered mullein flower infusion, one day before the control seeds. As shown by our study, the highest dynamics of germination was observed for seeds sown into soil pre-treated with the plant extract.

a) b)

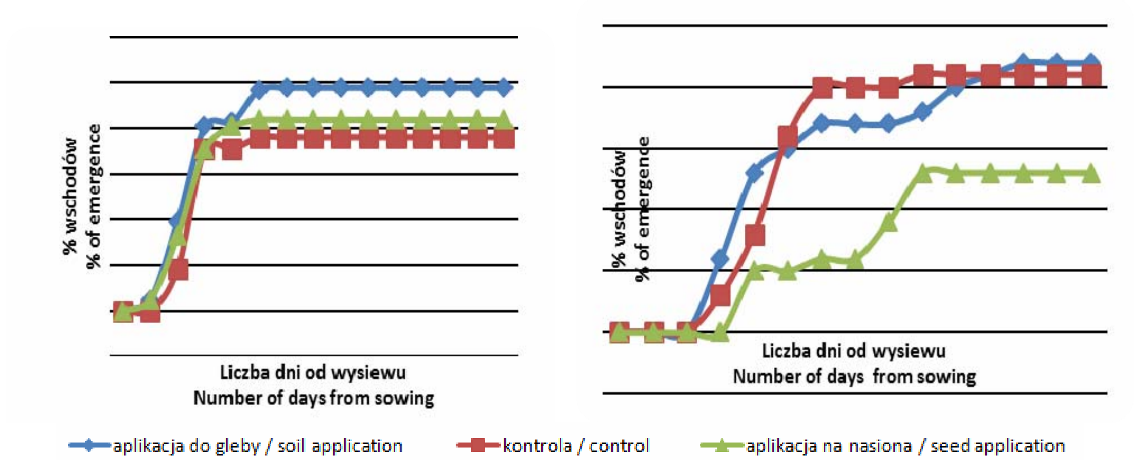


Source: own work / Źródło: opracowanie własne

Fig. 1. Dynamics of emergence of yellow lupine depending on the method of shaping the sowing seeds environment: a) seeds from the ecological farming, b) the soil 21 days after application of the preparation with glyphosate

Rys. 1. Dynamika wschodów łubinu żółtego w zależności od aplikacji: a) gleba z upraw ekologicznych, b) gleba po 21 dniach od zastosowania preparatu glifosatu

a) b)



Source: own work / Źródło: opracowanie własne

Fig. 2. Dynamics of emergence pea depending on the method of shaping the sowing seeds environment: a) seeds from the ecological farming, b) the soil 15 days after application of the formulation with glyphosate

Rys. 2. Dynamika wschodów grochu siewnego w zależności od aplikacji: a) gleba z upraw ekologicznych, b) gleba po 21 dniach od zastosowania glifosatu

The dynamics of field pea seed germination in the conventionally cultivated soil was different (Fig. 2b). In this environment, seed germination was more extended in time. First seedlings were observed as late as after 4-5 days. The mean number of seedlings in the conventionally cultivated soil was significantly reduced: germination capacity was lowered by a factor of more than 6 for the combination involving direct application of plant extract into the soil, more than 3 for the control seeds, and more than 4 for seeds pre-treated with plant extracts as compared to the respective results in ecologically cultivated soil. Similar observations were made by Piotrowicz-Cieślak et al. [9]. The authors found that germination of garden cress, white mustard, sorghum bicolor, or yellow lupine was reduced by 15% in soils that had been previously treated with glyphosate. Also the studies conducted by Sikorski et al. [13] showed that glyphosate reduced the lengths of the roots and stems of yellow lupine seedlings. The authors also demonstrated that the in-field concentration of glyphosate as recommended by the manufacturer was phytotoxic to the seedlings of yellow lupine.

4. Conclusions

Application of garlic bulb or dense-flowered mullein flower infusions contributed to the increased germination capacity of yellow lupine and field pea seeds, respectively, as compared to the control seeds. Significantly the highest number of seedlings was observed in the case of direct soil administration of the tested plant extracts.

The lowest germination capabilities were observed for yellow lupine and field pea seeds sown into conventionally cultivated soils that had been treated with glyphosate-containing herbicide 21 days earlier.

The highest and the most uniform germination rates were observed for field pea seeds in ecologically cultivated soils following the use of both methods of plant extract pre-treatment.

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