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BIOSTIMULATING EXTRACTS FROM *ARCTIUM LAPPA* L. AS ECOLOGICAL ADDITIVES IN SOYBEAN SEED COATING APPLICATIONS

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ABSTRACT

This paper proposes a new biostimulant coating for soybean seeds. The aim of the study was to create a coating for *Glycine max* (L.) Merr. soybean seeds, using root infusion from *Arctium lappa* L. as a biostimulant component. The effectiveness of the produced coating was evaluated in a three-year field study. The analysis of the effectiveness of the developed coating was based on the evaluation of plant biometric traits and yield. The study showed that the designed and manufactured soybean seed coating based on the root infusion of *Arctium lappa* L. can be considered as a new agronomic strategy to improve the productivity of soybean *Glicyne max* (L.) Merr. under actual field conditions. The application of the biostimulant coating resulted in soybean plants with significantly increased biometric traits (plant height, height of the first pod set, number of pods per plant, number of seeds per pod) and productivity (yield improvement of more than 10%). Only a reduced weight of 1,000 seeds compared to control samples was noted.

Introduction

The application of seed coatings to crop seeds is an agricultural practice used not only to protect seeds, but also to increase germination and plant emergence. Applying exogenous materials to the surface of the natural seed coat leads to changes in the physical properties of the seed (Avelar et al., 2012) and can be a source of active ingredients. A properly created seed coating can be considered as a matrix for various bioactive compounds, including biostimulants (Salanenka and Taylor, 2006; Salanenka and Taylor, 2011).

There are a number of requirements for the materials used to design a seed coating, including compatibility with active compounds and biocompatibility with physiological processes. The materials must not prevent seedgermination and seedling growth in any way

(Pedrini et al., 2017). However, please note that seed treatments will show the greatest effectiveness when they address crop-specific needs. This results in increased yieldsunder changing climatic conditions, in line with sustainable agriculture (Williams et al., 2016).

Maximization of crop yields while using fertilizers sustainably is one of the greatest challenges ofmodern agriculture. This is why biostimulants have been gaining interest recently (Farias et al., 2019). This group of agricultural formulations includes plant extracts that observably improve the growth and development of crops, but do not exhibit a fertilizer-like effect. This is because they affect the regulation of metabolic processes in plants.

The use of biostimulant components based on plant extracts has not yet been widely used in seed treatments in agriculture (Afzal et al., 2020), but the development of natural and non-toxic seed coating additives has gained importance nowadays because of their application in organic agricultural systems. Plant extracts are environmentally friendly, low-priced, and relatively easily available ecological additives that improve coating performance (Maria et al., 2016). The source of extracts can be various morphological parts of plants (roots, fruits, seeds, leaves) (Hajar et al., 2016).

The efficacy of biostimulants is largely due to their antioxidant potential (Singh et al., 2017) and the content of phenolic compounds, which show positive effects on plant growth and development at low concentrations. Plant extracts, as part of the designed seed coating, are described as phytoactive promoters, with the potential to shape seedling vigor and increase resistance to biotic and abiotic stresses. Thus, new solutions for the delivery of bioactive compounds through seed coating seem crucial for sustainable agriculture and restoration ecology (Ong et al., 2021). Successful innovation in plant extract development also requiresthe optimization of extraction methods, including the solvents used. Among solvents, methanol, acetone or ethanol are often used, however, hydroextraction seems more advantageous from the point of view of possible ecological applications. The choice of the right solvent is also of great importance for the functional properties of seed coatings, which can exhibit antibacterial or antifungal activity in addition to the stimulant effect (Pedrini et al., 2017).

Purpose and scope of work

The objective of the study was to create a coating for *Glycine max* (L.) Merr. soybean seeds, using root infusion from *Arctium lappa* L. as a biostimulant component. Evaluation of the effectiveness of the created coating was evaluated in a three-year field trial, which allowed for considering the hypothesis of improved biometric traits and yield of soybean plants.

Methods of work

Preparation of the infusion

The plant extracts were prepared by hot water extraction from dried, ground roots of *Arctium lappa* L. (Runo Poland, PL-EKO 04 EU Organic Farming). The infusions were prepared according to the method given by Biegański (1950). 10 g of ground roots were added to 250 mL of distilled water. The resulting solution was kept at 100ºC in a water bath for 30 minutes. The extract was centrifuged at 4250 rpm for 5 minutes and filtered through Whatman No. 1 filter paper.

Creation of soybean seed coating

The soybean seed coatingwasprepared based on the method described by Rajapaksha and Shimizu (2021), with own modifications. The coating was made by mixing starch from *Manihot esculenta* Crantz (2 g of starch) with 1 g of glycerol and 0.1 g of lemon pectin. The ingredients were heated at 85ºC on a magnetic stirrer until the starch gelatinized. The solution was then cooled and 88.0 g of burdock (*Arctium Lappa* L.) root infusion was added. The resulting coating was applied onsoybean seeds of *Glicyne max* L. 'Abelina' variety in a seed dressing machine.

Testing the effectiveness of the coating in field trials

The effectiveness of coatings was tested in a 2016-2018 field experiment conducted in the village of Perespa (50º66'N; 23º63'E, Poland). The area of experimental plots was15 m^2, and the soil used was alkaline (pH in 1M KCl: 7.3-7.4).The experiment was carried out in 4 replicates, in a randomized block system. Coated soybean seeds and control seeds were sown in 2016, 2017 and 2018 at 4.0 cm intervals in rows with 30 cm spacing (Szparaga et al., 2018).

In each year of the experiment, 25 plants were taken randomly from the plots. They were evaluated for biometric traits (plant height, height of the first pod set, number of pods per plant, number of seeds per pod, thousandseed weight) and their yield was also determined.

Statistical analysis

All analyses were performed in triplicate. The Shapiro-Wilk test was used to assess the normality of the data distribution. Results were analyzed using one-way analysis of variance (ANOVA). The significance of the differences between the mean values was evaluated using Tukey's confidence intervals, with a significance level of $p < 0.05$. Statistical analysis was performed using Statistica 13.3 software (TIBCO Software Inc., USA).

Research results and discussion

Previous studies on the use of burdock root infusion showed that it was abundant in phenolic compounds, the total pool of which was over 240 µg mL^{-1}. Fourteen phenolic compounds were identified in the infusion, among which the highest concentration was found for 1,4-dicaffeoyl-3-maloylquinic acid, 3,4-dicaffeoyl-5-succinoylquinic acid, chlorogenic acid and 1,3-dicaffeoyl-4,5-dimaloylquinic acid. The analysis of the chemical composition of burdock root infusion also showed carbohydrate content in the form of sucrose, glucose and fructose (Szparaga et al., 2021).

It should be concluded that burdock root infusion represents a complex system of bioactive compounds that will determine the effectiveness and efficiency of the coating applied to soybeans. According to Pedrini et al. (2017), the phytoactive promoters contained in the biostimulant extracts are biologically active. The method of their application in seed coatingwill determine the regulatory processes of plant cell growth, division, or development (Rolland et al., 2006; Szparaga et al., 2021).

The three-year field experiment conductedto evaluate the actual effects of the produced coating showed that the initial growth and development of these crops is a determinant of their final yield potential. The evaluation of biometric traits showed that soybean plants grown from coated seeds exhibited significantly greater height in relation to control samples (uncoated seeds) (Fig. 1). Observation of the average effects in the field experiment showed that the plants grown from coated seeds were more than 13% higher compared to the control samples.

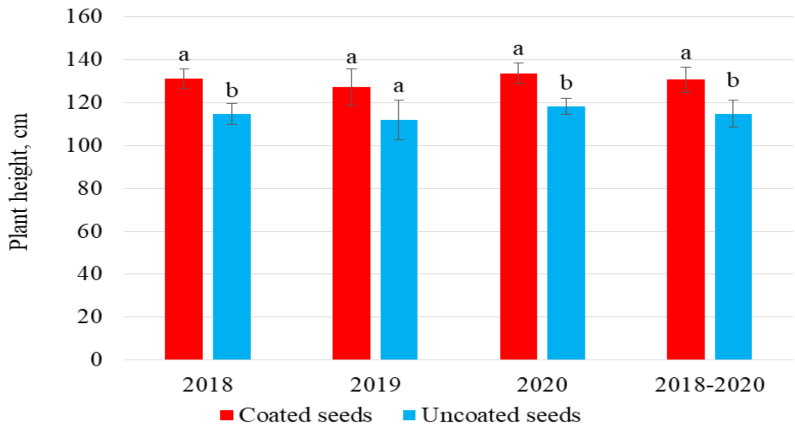

Figure 1. Height of soybean plants, from crops where coated seeds and control seeds were sown

Similar trends were observed when evaluating the setting of the first pod and the number of pods per plant (Fig. 2-3).

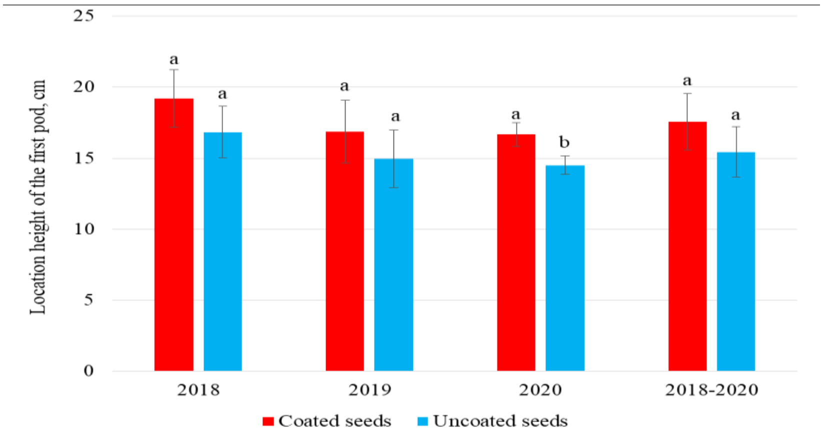

Figure 2. Height of first pod set on soybean plants from crops where coated seeds and control seeds were sown

The height of setting of the first pod in soybean plants for which coated seeds were used for sowing increased by more than 13% compared to plants from control seeds. This biometric parameter is particularly important for combine harvesting of soybeans. In contrast, sowing coated seeds resulted in an increase in the number of pods per plant by an average of about 18%.

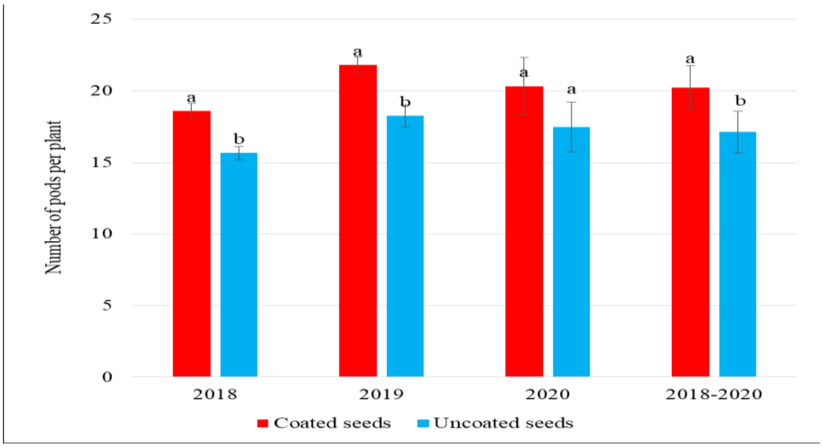

Figure 3. Number of pods per plant for soybeans from crops where coated seeds and control seeds were sown

In addition, it was observed that the effect of the biostimulant coating was a significantly increased number of seeds per pod (by more than 14% on average) (Fig. 4).

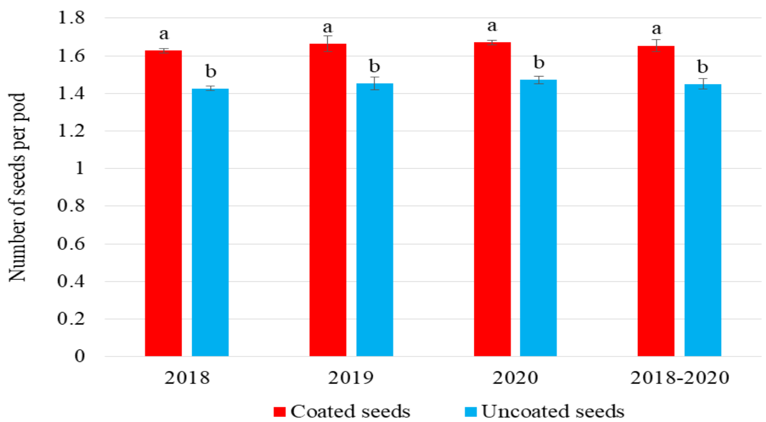

Figure 4. Number Number of seeds per pod from soybean plants from crops where coated seeds and control seeds were sown

The only adverse effect observed was a reduced weight of 1,000 seeds compared to control samples (an average decrease of approximately 15%) (Fig. 5).

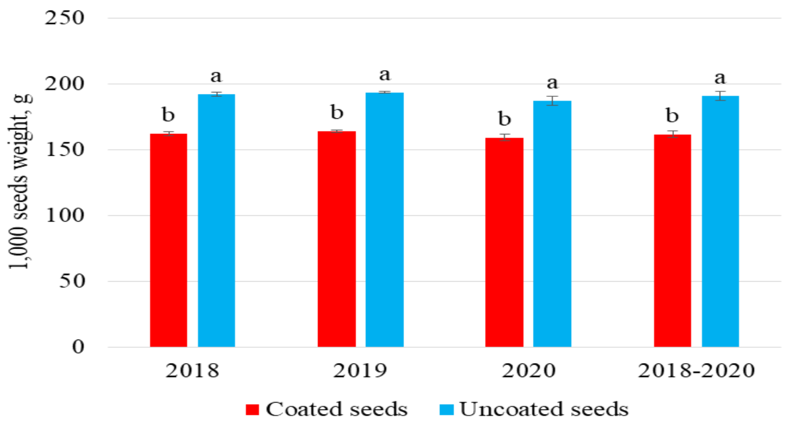

Figure 5. The weight of one thousand seeds from soybean plants from crops in which coated seeds and control seeds were sown

However, from the point of view of the practical applicability of the biostimulant coating, the most important thing is to improve the yield-forming capacity of plants. Field tests showed that the application of the new coating, based on an infusion of burdock root, had a biostimulatory effect on plant growth and development, resulting in increased soybean yield (Fig. 6). This increase was observed in each year of the study, compared to the control (by almost 14% on average), which allowed us to accept the hypothesis of a positive effect of the bio-coating on soybean seeds, under actual field conditions.

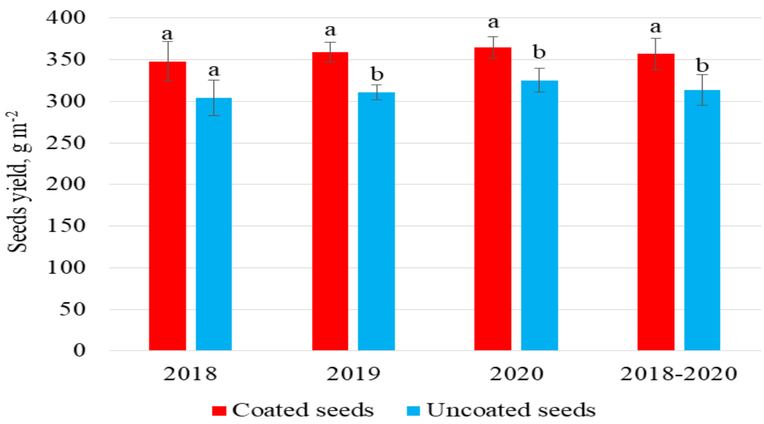

Figure 6. Soybean seed yield, crops from coated seeds and control seeds are compared

The reported results of the studycan be explained by the composition of the new coating, which was rich in polyphenolic acids and carbohydrates. According to Salwa et al. (2011), such a coating composition determines plant growth and yield. The possible synergistic interaction of bioactive components inthe burdock rootinfusion plays an important role in cell division, meristematic cell development, as well as photosynthesis and respiration processes (Zeidan et al., 2010).

It should also be emphasized that polyphenolic compounds contained in the infusion of burdock root could exhibitan auxin effect, i.e., inappropriate concentrations, it stimulates plant growth and development. This is due to the fact that phenolic compounds affect the hormonal balance of plants (hormone biosynthesis). In addition, a hypothesis has been presented that some of the polyphenols, which penetrate the cell membrane, can activate the production of secondary metabolites intracellularly. However, the mechanism of phenolic compounds on the cell is still unidentified (Tanase et al., 2019).

In addition, the improvement in plant development and yield could be the result of antimicrobial activity of burdock extracts, thereby protecting germinating seeds and developing seedlings from the negative effects of at least fungi, present in the soil. The use of the infusion in the coating couldhave additionally activated a number of detoxifying enzymes and transporters. As a result, toxins from developing plants were deactivated and eliminated, and adaptation processes of the relevant metabolic processes were induced (Pedrol et al., 2006). According to Amirkhani et al. (2019), from the point of view of crop yield, the use of biostimulants as seed coatings is more cost-effective and efficientthan its foliar or soil application. In addition, Amirkhani et al. (2016) showed that coatings with a biostimulant component improve germination and stimulate growth of not only agricultural but also horticultural crops. A study by Qiu et al. (2020) proved that seed coating with biostimulants resulted in increased germination of red clover and ryegrass. According to Afzal et al. (2020), the observed effects ofseed coatings are related to increased nutrient uptake from both the seed coat and the soil. A study by Lim et al. (2020) showed that applying biostimulants to sweet corn seeds significantly improved the biometric traits and yield of these plants. According to the researchers, seed coating promotes rapid plant development at early stages, and thus can enhance plant growth at later stages.

The observed improvement in biometric traits and soybeanyield after the application of seed coatings could also be related to the presence of carbohydrates in the infusion of burdock roots. In fact, carbohydrates are considered regulators of many growth, developmental, or metabolic processes, regardless of their primary functions. Therefore, it can be assumed that, in addition to phenolic compounds, the concentration of carbohydrates in the seed coat will determine its biological activity in thebiostimulation of plant development, affecting the primary antioxidant system of seeds and developing seedlings. This is of particular importance in supporting plants against stress factors (Hayat et al., 2018).

Savvides et al. (2016) and Gupta et al. (2022) proposed that seed coating is a key agronomic practiceto improve seed germination and increasing seedling vigor, which consequently contributes to improving crop production efficiency and increasing yields under optimal and suboptimal conditions. Meanwhile, creating seed coatings based on natural biostimulants is currently one of the most innovative and promising strategies for sustainable agriculture (Barone et al., 2018).

Conclusions

1. The research proved that the designed and manufactured soybean seed coating based on *Arctium lappa* L. root infusion can be considered as a new agronomic strategy to improve the productivity of soybean *Glicyne max* (L.) Merr. under actual field conditions.
2. The application of the biostimulant coating resulted in soybean plants with significantly increased biometric traits (plant height, height of first set of pods, number of pods per plant, number of seeds per pod) and productivity (yield improvement of more than 10%). The only adverse effect observed was a reduced weight of 1,000 seeds compared to control samples.
3. However, despite the promising results of the field trials, further research must be carried out to clarify the mechanism of plant response to the applied biostimulant coatings, which will allow the development of the design of innovative coatings for crop seeds.

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BIOSTYMULUJĄCE EKSTRAKTY Z *ARCTIUM LAPPA* L. JAKO EKOLOGICZNE DODATKI DO POWLEKANIA NASION SOI

Streszczenie. W pracy zaproponowano nową powłokę biostymulującą dla nasion soi. Celem badań było stworzenie powłoki dla nasion soi *Glycinemax* (L.) Merr. z wykorzystaniem naparu z korzenia *Arctiumlappa* L. jako komponentu biostymulującego. Skuteczność wytworzonej powłoki oceniono w trzyletnich badaniach polowych. Analizę efektywności opracowanej powłoki oparto na ocenie cech biometrycznych roślin oraz plonu. Badania wykazały, że zaprojektowana i wyprodukowana powłoka do nasion soi na bazie naparu z korzenia *Arctiumlappa* L. może być traktowana jako nowa strategia agronomiczna poprawiająca produktywność soi *Glicyne max* (L.) Merr. w rzeczywistych warunkach polowych. W wyniku zastosowania powłoki biostymulującej uzyskano rośliny soi o istotnie zwiększonych cechach biometrycznych (wysokość roślin, wysokość osadzenia pierwszego strąka, liczba strąków na roślinie, liczba nasion w strąku) i produktywności (poprawa plonu o ponad 10%). Odnotowano jedynie obniżoną masę 1000 nasion w stosunku do prób kontrolnych.

Słowa kluczowe: ekstrakt, powłoka, biostymulant, plon