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Evaluation of the Effects of Allelopathic Aqueous Plant Extracts, as Potential Preparations for Seed Dressing, on the Modulation of Cauliflower Seed Germination

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Abstract: Allelopathic plants can be widely used in bio-farming considering their potential role in the improvement of seed germination. The study presented in this work was designed to evaluate the effects of two extraction methods and the effects of allelopathic aqueous extracts from twenty plants as seed dressing preparations on the number of germinating and infested seeds of cauliflower (*Brassica oleracea convarietas L. botrytis var. botrytis*). Plant extracts (in the form of cold-soaked macerates and infusions) were used for seed dressing. The percentages of normally germinating, non-germinating, and pathogen-infested seeds were determined in a paper test. Of the 20 herbal plant species used in the study, the biopreparations extracted from *Zea mays* L. moles were the most effective as they evoked the most beneficial effects on both seed germination and reduction of infestation by microbial pathogens. The study also showed that infusions used for seed treatment were better at improving cauliflower seed germination than were macerates. This method of extract preparation probably enabled an increase both in the availability and activity of allelochemical compounds.

Keywords: cauliflower; allelopathy; germination; effectiveness; extracts; seeds

1. Introduction

The term allelopathy refers to the direct or indirect (adverse or beneficial) effect of one plant on the other as a result of producing and releasing metabolites to the environment [1,2]. Like all other compounds of the bio-systems, these metabolites are classified to two large groups. The first one, called major metabolites, includes chemical substances that determine growth and development, such as carbohydrates, amino acids, proteins, and lipids. The other one includes secondary metabolites, the main task of which is to aid plants by increasing their general ability to survive and withstand inconvenient environmental conditions [3]. It needs to be emphasized that, usually, the bioactive

compounds of plants are synthesized in the form of secondary metabolites [4], with the involvement of certain limited taxonomic groups of microorganisms. These compounds are characterized by exceptional and often complicated chemical structures and, additionally, are usually produced in the form of mixtures of closely related members of the same chemical family [5–7].

The secondary metabolites exhibiting allelopathic potential, referred to as allelochemicals, have been a focus of interest for many researchers over recent years [8–11]. Equally great attention has been paid to the phenomenon of allelopathy, since evidence has been found for its significant role in regulating the growth of plants and the development of agricultural systems, including the quality and size of the crop yield produced [1,2,12–14].

The knowledge and exploitation of allelopathy principles are especially useful in modulating seed germination. The formulation of appropriate preparations that would stimulate this most important stage of plant development is extremely important from the viewpoint of their practical application in both sustainable and ecological farming. Many studies have already confirmed that wet seed treatment not only improves germination rate and uniformity [15,16], but also stimulates various changes in seeds at the biochemical level that are necessary in breaking seed dormancy, activating enzymes, and aiding embryonic tissue development [17,18]. This allows for faster and more uniform emergence of plants [19], probably due to the enhanced mobilization of metabolites and sprouts, which consequently leads to growth promotion [20,21]. The literature on the subject provides some information about methods for improving seed germination and plant emergence involving the use of various chemicals, salts, and plant growth regulators [22–24]. However, results of multiple studies have shown these techniques to be costly and in many cases non-implementable on a large scale. Considering the above, a need has emerged to search for the agents of natural origin to be used for seed treatment, which would also contribute to a dynamic development of ecological agriculture. This task may be accomplished through studies of not only the allelopathic potential of plants but also of methods suitable for the extraction of their biologically active compounds. Only this approach would make it possible to formulate preparations applicable in agriculture. Due to the fact that a plant matrix is a rich and extraordinarily complex system, it is believed that there is no one and universal method for the extraction of all secondary metabolites from plants. Often, additional stages of sample preparation may be necessary. Prior to extraction, the plant material is usually subjected to processes such as lyophilization, air drying, disintegration, or grinding. Common methods used to extract biologically active compounds from plant materials include maceration, solvent extraction, extraction in a Soxhlet apparatus, and ultrasound- or microwave-assisted extraction. It needs to be emphasized that the effectiveness of extraction of small-molecular-weight compounds is strongly affected by process parameters, i.e., time and temperature. Temperature increase can, theoretically, facilitate solubilization of analytes and accelerate the process, however, such conditions may contribute to the hydrolysis or oxidation of most of the thermolabile compounds [25]. Therefore, extraction methods and parameters, including solvent type, determine the efficacy of the preparation in seed dressing due to their impact on the allelochemical substances of plants. The major compounds of this type, with documented allelopathic activity (including antioxidative and antimicrobial activities), include phenolic compounds with their important subclasses of phenols, phenolic acids, quinones, flavones, flavonoids, tannins, and coumarins [26,27]. Nevertheless, as indicated by many authors, the extraction process itself as well as biological trials with the extracts obtained will not be effective without understanding that the secondary plant metabolites indeed stimulate plant germination and growth, however, in the majority of cases, only at low concentrations [28,29]. This is due to the fact that most of the allelochemicals are phytotoxic in high concentrations [30–34].

Considering the fact that allelopathic plants can be widely used in both sustainable and ecological agriculture, this study was designed to evaluate the effects of two extraction techniques and the effects of allelopathic aqueous extracts from twenty plants used for seed dressing on the number of germinating and infested seeds of cauliflower (*Brassica oleracea convarietas L. botrytis var. botrytis*). A research hypothesis was advanced that cauliflower seed treatment with extracts potentially containing

multiple plant growth regulators, mineral nutrients, and vitamins can promote plant growth at the germination stage.

2. Materials and Methods

The research material consisted of seeds of cauliflower (*Brassica oleracea convarietas* L. *botrytis* var.) of 'Bering F1' cultivar and herbal plant species from which biological samples were obtained in the form of infusions and macerates. The following 20 species of dried herbal plants were used: *Sambucus nigra* L. (flowers), *Betula verrucosa* Ehrh. (leaves), *Artemisia sativum* L. (bulb), *Allium sativum* L. (bulb), *Aesculus hippocastanum* L. (bark), *Aesculus hippocastanum* L. (flowers), *Mentha piperita* L. (leaves), *Saponaria officinalis* L. (root), *Urtica dioica* L. (leaves), *Equisetum arvense* L. (herb), *Marrubium vulgare* L. (herb), *Acorus calamus* L. (rhizome), *Crataegus oxyacantha* L. (flower), *Frangula alnus* Mill. (bark), *Zea mays* L. (moles), *Melissa officinalis* L. (leaves), *Taraxacum officinale* Web. (root), *Inula elenium* L. (root), *Matricaria chamomilla* L. (baskets), and *Rosa canina* L. (fruit).

The above presented plant material included common herbal plants grown in Poland and Europe. They were chosen for the study considering their rich and varied chemical composition and, most of all, due to the high number of compounds with germination-stimulating potential. Due to the fact that the harvest time of the selected plants differed extremally, dried plant material was purchased at the Zakład Konfekcjonowania Ziół FLOS (Poland), and ground to a fraction size of 500 µm [35].

Extracts were produced using dried and ground parts of 20 plants. Two extraction methods were used, based on modified procedures provided by Sas-Piotrowska and Piotrowski [36] and Godlewska et al. [37], respectively: (1) A hot extraction method in the form of infusions, by adding 5 g of plant material powder to 250 mL of distilled water and boiling the mixture in a water bath for 30 min; and (2) a cold-soaking method in the form of macerates by adding 5 g of dried plant material to 100 mL of distilled water and leaving the solution in a dark and cold room for 48 h. Afterwards, the extracts produced with both methods were centrifuged at 4250 rpm for 5 min and filtered through Whatman no. 1 filter paper.

Germination capacity analysis was performed as a tissue test based on ISTA [38] standards (International Seed Testing Regulations: Chapter 7). The experiment aimed to determine the percentage of germinated seeds, non-germinated seeds, and seeds infested by microorganisms. The results obtained were compared with the control, which consisted of seeds treated with sterile water. Each of the combinations consisted of 100 seeds and the tests were performed in four repetitions. The analysis of variance of the results obtained was performed using Tukey's test at the significance level of $\alpha = 0.05$. The standard deviation (SD) value was determined for all reported mean values.

3. Results and Discussion

The analysis of variance showed that the percentage of germinated and not-germinated seeds was significantly influenced by the plant species used to prepare aqueous extracts (Figure 1, Figure 2). However, no statistically significant differences were found based on the production method of the biopreparations. Interactions between the studied traits were also insignificant (Figure 1).

From among all analyzed plant species, 35% inhibited germination of the studied material, which resulted in obtaining more nonsprouting seeds in relation to the control object. Germination of seeds was inhibited to the greatest extent by extracts obtained from *Matricaria chamomilla* L. (12.42%) and *Inula elenium* L. (14.10%).

Ismail et al. [39] presented the potential use of extracts with germination-inhibiting properties. They analyzed the oil obtained from *Juniperus oxycedrus* L. and showed its effectiveness in inhibiting the growth of seedlings of three weeds: *Phalaris paradoxa*, *Trifolium campestre*, and *Lolium rigidum*.

Although no significant differences were found depending on the preparation method of extracts, 65% of the macerates obtained were more effective in seed germination control, especially flowers of *Aesculus hippocastanum* L. (4.80% of nonsprouted seeds), *Frangula alnus* Mill. (4.80%), and *Zea mays* L. (5.00%). Only the treatment of the tested seed with macerate from *Allium sativum* L. resulted in the

analyzed parameter being at the same level as in the control combination. However, the remaining 30% of the macerates obtained caused an increase in the number of nongerminating seeds compared to the control.

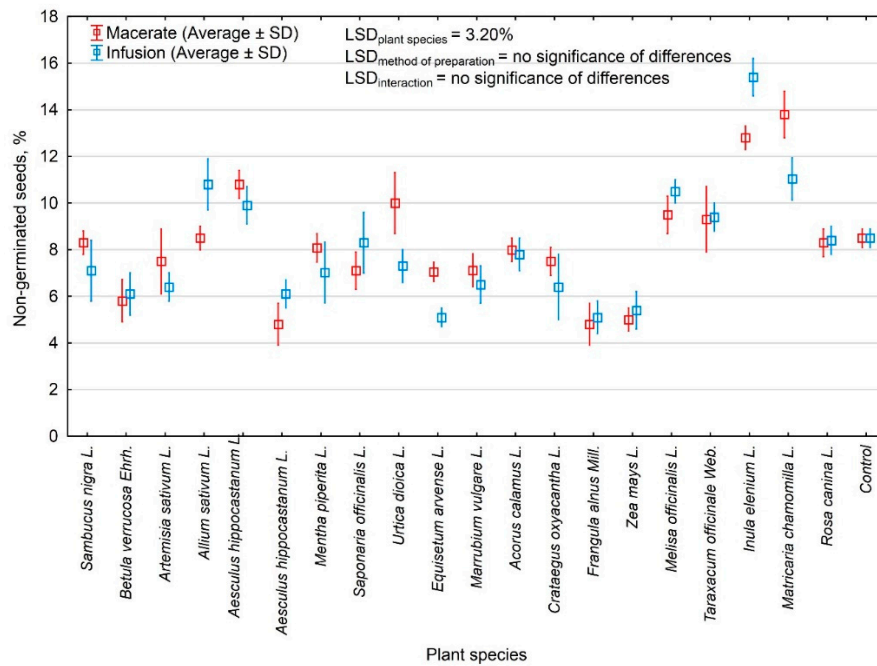


Figure 1. Nonsprouted seeds (%) depending on the plant species from which the extracts were obtained and the method of extraction. SD: standard deviation, LSD: less significant difference.

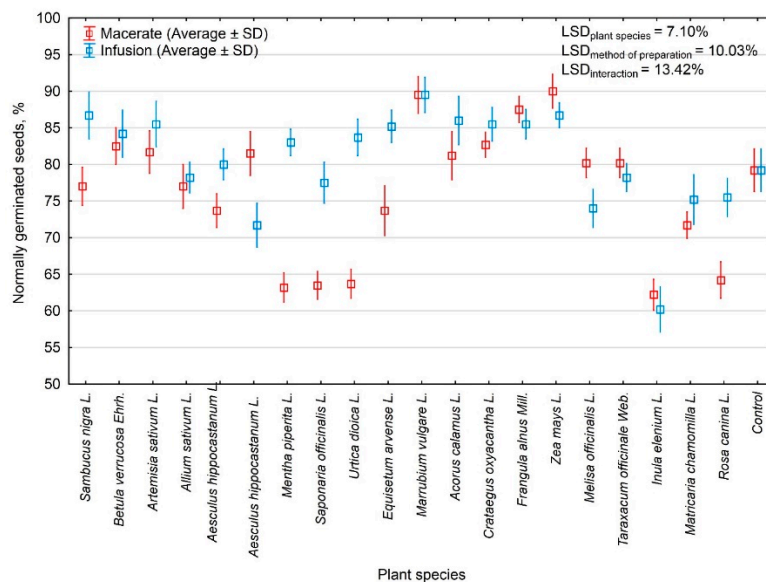


Figure 2. Normally sprouted seeds (%) depending on the species of plant from which the extracts were obtained and the method of extraction. SD: standard deviation, LSD: less significant difference.

Infusions were slightly more effective than macerates, as 70% of them decreased the number of nonsprouted cauliflower seeds. The strongest were *Equisetum arvense* L. and *Frangula alnus* Mill. In both cases, the percentage of nonsprouted seeds was on average 4.80%. The macerate obtained from the roots of *Inula elenium* L. was the least effective in this respect as about 15.40% of unsprouted seeds were obtained in its case. Munir and Tawaha [40] showed that, apart from the extraction method, the morphological part of the plant from which the extract is obtained was also of significant importance.

They observed that aqueous preparations obtained from *Brassica nigra* L. leaves limited the germination of lentil seeds to the greatest extent in relation to the extracts obtained from flowers, roots, and stems. Additionally, Filippo-Herrera et al. [41] showed a high effectiveness of seaweed extracts in treatment of mung bean seeds. Brown algae extracts were more effective than red algae extracts.

No surface contamination of the tested material by fungal microorganisms was found. The cauliflower seeds were infested only by bacterial pathogens. However, the analysis of variance showed that the herbal plant species, the method of obtaining the biopreparations, and the first-order interaction did not significantly affect the colonization of the analyzed material by the microorganisms. The lowest contamination of seeds with pathogens was observed in the combination of *Frangula alnus* Mill extracts used as a seed dressing (average 3% of infested seeds). In turn, the plant species whose preparations contributed to the highest contamination was *Allium sativum* L. The growth of microorganisms on the seed surface in relation to the control was about 1.25%.

Analyzing the effectiveness of macerates in the context of limiting the contamination of the examined material with microorganisms, it can be concluded that only 35% of them exhibited such properties. In both cases, the percentage of infested seeds was 2.50% on average (Figure 3). Treatment of the tested material with macerate obtained from *Sambucus nigra* L. was characterized by the highest infestation by pathogens, causing 5.80% of infested grains on average.

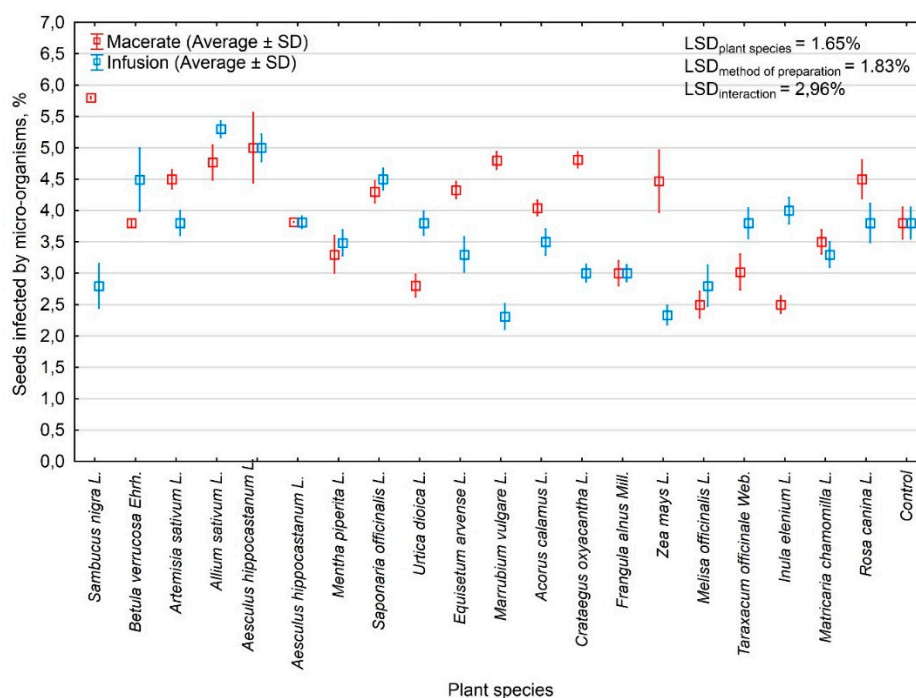


Figure 3. Seeds infected by microorganisms (%) depending on the species of plant from which the extracts were obtained and the method of extraction. SD: standard deviation, LSD: less significant difference.

The use of infusions as a seed dressing resulted in 50% reduction of cauliflower seeds infestation by microorganisms, especially when prepared from *Marrubium vulgare* L. and *Zea mays* L. The average number of seeds infested by pathogens in each of these cases was 2.30%. Infusions obtained from flowers of *Aesculus hippocastanum* L., roots of *Taraxacum officinale* Web., bulb of *Artemisia sativum* L., leaves of *Urtica dioica* L., and fruits of *Rosa canina* L. did not inhibit or promote the presence of microorganisms on the seed surface, and the obtained value of the analyzed parameter was at the level of the control object. The highest contamination of seeds occurred after the application of infusions from bulb of *Allium sativum* L. (5.30% of infected seeds) and bark of *Aesculus hippocastanum* L. (5.00% of infested seeds).

In summary, it needs to be emphasized that the available literature provides little information on the use of allelochemical extracts for seed dressing. However, the use of such preparations proved they can stimulate germination of cauliflower seeds and, at the same time, decrease the number of seeds infested by microorganisms, which in practice will result in improved plant growth and productivity. In their investigations, Afzal et al. [42] and Pill and Savage [43] demonstrated that seed treatment with an extract from sugar beet improved seeds germination by ensuring their physiological superiority. Results of our study showed that extraction methods significantly influenced the efficacy of the extracts obtained. Differences in their activity could be due, most of all, to extraction process parameters that have various effects on the softening and destruction of a plant cell wall and the release of soluble phytochemicals. Hence, the extracts could differ also in their antioxidative potential, which in turn might either enhance or suppress the activity of such free-radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in cauliflower seeds [44]. These enzymes improve the plant's capability for maintaining an appropriate growth rate [45,46]. As mentioned earlier, plants are rich sources of antioxidants, minerals, or vitamins that may potentially stimulate crop seed germination. Basra et al. [47] reported an increase in sprouting and emergence potential, and also in phenols content in maize seedlings when maize seeds were treated with plant extracts. According to these authors, an increase in phenols content upon seed dressing can be ascribed to a higher content of bioactive compounds. Yasmeen et al. [20] demonstrated that seed treatment also improved vitality of seedlings and enhanced the activity of scavenging enzymes in wheat leaves (increased activities of SOD, POD, and CAT, and increased total phenolic contents (TPC)). This was also confirmed by results obtained by Basra et al. [47,48], who showed that seed treatment with allelochemical extracts significantly enhanced activities of CAT and SOD in plants. Therefore, it is likely that the activity of the antioxidative enzymes in cauliflower seeds enhanced by seed treatment with botanical extracts could be due the high content of their antioxidants.

Results of our study also indicate that the allelochemical extracts contributed to a decreased number of cauliflower seeds with manifestations of infestation by pathogens. This can be a valuable clue in developing alternative control strategies to reduce dependence on synthetic fungicides used for seed dressing. Study results confirm that the plant materials tested were rich in aromatic secondary metabolites that exhibit antimicrobial activity, as many authors have emphasized that components with phenolic structures, such as carvacrol, eugenol, and thymol, are highly active against plant pathogens. Hence, these groups of compounds serve as the protective mechanisms of plants against pathogenic microorganisms [49]. This was confirmed by results reported by many authors, for example, an antimicrobial substance, allicin (diallyl thiosulfinate), present in garlic extracts was found effective in controlling the development of *Alternaria* spp. on carrot seeds, and of *Phytophthora* leaf blight on tomato seeds [50]. However, such research has just begun. Today, scientists search for and investigate plant extracts with antimicrobial properties. To make the search for new biologically active plant products more systematic, it will be necessary to standardize extraction methods and the methods used to analyze the antimicrobial effectiveness in vitro and then in vivo, in order to assess the effectiveness of these products in controlling plant disease incidence at each stage of plant phenological development [26].

4. Conclusions

Cauliflower seeds showed varied responses to allelopathic aqueous plant extracts. The study demonstrated that seed viability was largely dependent on the plant species from which the preparations had been made, method of their production, and interactions between these factors.

The highest numbers of normally-sprouting cauliflower seeds were reported after seed treatments with the extracts from *Zea mays* L. and *Marrubium vulgare* L., whereas the lowest percentages of non-sprouting seeds were achieved upon the use of the extracts from *Frangula alnus* Mill, *Zea mays* L., and flowers of *Aesculus hippocastanum* L.

In turn, seed infestation by microorganisms was most effectively reduced by the extracts obtained from *Frangula alnus* Mill., *Zea mays* L., and *Aesculus hippocastanum* L.

Of the 20 herbal plant species used in the study, the most effective turned out to be the biopreparations extracted from moles of *Zea mays* L. because they had the most beneficial effect on all parameters studied.

Study results enable us to conclude that the use of infusions for seed dressings yielded better effects in terms of cauliflower seed germination. Most likely, this method of extract production allowed for an increase in both the availability and activity of allelochemical compounds.

To summarize our study on the effects of allelopathic plant extracts, we not only demonstrated that they stimulated cauliflower seed germination and reduced seed infestation by pathogenic microorganisms but we also showed their extremely important practical applicability connected with the feasibility of producing and implementing novel and natural seed dressings.

Taking into account the promising results we have demonstrated in this work, we are aware that their implementation in sustainable and organic agriculture in the future will still require extensive research. The first task of future studies will be the in-depth characterization of the chemical composition of the extracts tested, which will be followed by a change of the experimental scale, because only real conditions in field experiments will allow for the complete evaluation of the effectiveness of these allelochemical preparations.

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