

Article

# Evaluation of the Effectiveness of the Use of Biopreparations as Seed Dressings

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**Abstract:** In laboratory tests, the effectiveness of water plant extracts obtained from 20 species of herbal plants on the degree of contamination of white mustard seeds (*Sinapis alba* L.) by fungal and bacterial pathogens was evaluated. The analysis showed that the contamination of the tested seed material was statistically significantly influenced by the plant species from which the biopreparations were obtained. However, there were no significant differences in the method of preparation of extracts for the examined traits. The tested natural preparations had a different effect on limiting the superficial growth of bacteria and fungi on seeds. However, infusion and macerate obtained from the bark of *Salix alba* L. were the most effective in limiting the contamination with both bacterial and fungal pathogens. The number of seeds infected with fungi after the application of these preparations was 24.25% on average, whereas in the case of bacterial colonization of the tested material, the value of this indicator was 1.00% on average.

**Keywords:** bacteria; fungi; plant extracts; white mustard

## 1. Introduction

White mustard (*Sinapis alba* L.) is a cruciferous plant grown in many parts of the world. Its importance in agricultural cultivation is steadily increasing due to its multidirectional use for seeds, as a postharvest crop and as a melliferous plant. *Sinapis alba* L. cultivated after cereals as a catch crop plays the role of a phytosanitary plant, as it reduces the possibility of occurrence of pests and diseases of cereal plants. Being cultivated on weak soils may cause improvement of their chemical composition [1,2].

The agronomic value of white mustard lies in the seeds, which are currently used in food, pharmaceutical, cosmetic, chemical, and energy industries [3,4]. These seeds have a relatively low starch content and 25%–30% fat and 27%–35% protein with a very good amino acid composition. The seed coating constitutes about 20% of the seed mass [3,5]. Due to the chemical composition and taste of white mustard seeds, they are mainly used in the food industry to produce mustards, oil and spices [6].

However, consumers increasingly appreciate the production of agricultural crops with high nutritional and functional quality, in particular when they are produced using technologies with minimal environmental impact [7,8]. An important element in assessing the quality of food products by consumers is their safety, which is linked to the barrier to the presence of microorganisms and their secondary metabolites [9,10]. The fulfilment of these objectives can be met by the use of natural preparations stimulating growth and development of plants during cultivation. Biopreparations can also be used as seed mortars, increasing germination capacity and reducing contamination of seeds with pathogenic pathogens [11,12]. These agents may contain mixtures of active compounds (amino acids, proteins, polyphenols) and/or microorganisms.

Treatment of plants and seeds with biopreparations, apart from increasing the quality of yields and supporting growth and development of plants, does not affect further contamination of the ecosystem, which at present seems to be of key importance [13]. It is expected that only 3.5% of the world's land area is not subject to ecological restrictions [14]. This was mainly due to the excessive use of agrochemicals, which were used disproportionately to the actual needs of plants [15,16]. The focus is therefore increasingly on natural preparations as new agricultural technologies to protect and increase crop yields [17–19].

In addition, the use of biopreparations can reduce cultivation costs and increase the efficiency of the use of soil nutrients by plants, which in the end will reduce the incidence of diseases caused by nutrient deficiencies. However, these effects are not easy to achieve and require a lot of knowledge from the farmer about the selection of an appropriate preparation, the method of its application and the correct adjustment of doses and concentrations [19–22].

In agricultural crops, plant extracts can be used as biopreparations, but their effectiveness depends largely on the species of plant from which they are obtained. Additionally, the beneficial effect of plant extracts may vary both among species and varieties of agricultural plants [23]. Therefore, there is a need to study plant extracts in the context of their efficacy. Therefore, the aim of this study was to determine the possibility of using natural plant preparations as a seed mortar limiting the contamination of white mustard seeds (*Sinapis alba* L.) with bacterial and fungal microorganisms.

## 2. Materials and Methods

### 2.1. Plant Material

The plant material consisted of droughts of the following herbal plants: *Levisticum officinale* L. (root), *Coriandrum sativum* L. (fruit), *Pinus sylvestris* L. (young shoots), *Satureja hortensis* L. (herb), *Lavandula vera* L. (flower), *Linum usitatissimum* L. (seeds), *Quercus robur* L. (bark), *Arctium lappa* L. (root), *Calendula officinalis* L. (flower), *Juglans regia* L. (leaves), *Salix alba* L. (bark), *Origanum majorana* L. (leaves), *Archangelica officinalis* L. (root), *Ribes nigrum* L. (leaf), *Camelia sinensis* L. 'Pu-erh'. (leaves), *Artemisia absinthium* L. (herb), *Verbascum thapsiforme* L. (flower), *Hyssopus officinalis* L. (herb), *Juniperus communis* L. (fruit), and *Carum carvi* L. (fruit). The plants were used to obtain aqueous plant extracts, which treated the seeds of *Sinapis alba* L. of the Metex variety.

In the studies, plant preparations were produced in the form of aqueous extracts, macerates and infusions. Extracts were prepared according to the recipe given by Sas-Piotrowska et al. [24]. Macerate (cold method) was prepared from 5 g of dried fruit, which was then flooded with 100 mL of water at a temperature of about 20 °C, all of it was left for 24 hours at room temperature. The infusion (hot method) was obtained from 5 g of dried herbs, which were flooded with 250 mL of water at a temperature of about 100 °C and left covered for 30 minutes at a temperature of 20 °C. All the obtained plant extracts were filtered with filtration filters in order to obtain a clear preparation [21].

White mustard seeds were soaked for 24 hours at 20 °C in obtained biological preparations. The seeds were then placed in boxes lined with filter paper moistened with distilled water. Each box contained 100 seeds, covered with a single layer of filter paper moistened with 10 mL of distilled water. The health analysis of white mustard seeds consisted in the evaluation of their contamination

by bacterial and fungal pathogens on two dates: 3 and 7 days after the experiment was set up [25]. Seeds with signs of microbial infection were intended for further testing. A sample of colonizing microflora was taken from the seed surface. Identification of bacterial infection was made on the basis of microscopic observations. The ability to stain isolated bacterial cells was determined using the Gram method and spore staining with the Schaeffer–Fulton method. Identification of mold fungal infection was made on the basis of macro- and microscopic features, taking into account such morphological structures as: hypha, sporangia and spores as well as conidial stems, conidial syndrome or conidial spores [13,26].

## 2.2. Statistical Analysis

The conducted tests were performed in four repetitions for each plant extract. The control was performed on seeds soaked in distilled water. The analysis of the variance of the obtained test results was performed using the Tukey test at the level of significance  $\alpha = 0.05$ .

## 3. Results and Discussion

Plants and their products have been used by humans for centuries. The long tradition of their use results from their medicinal properties against pathogenic pathogens [27]. These properties have become a driving force in research on the use of plant extracts in agricultural practices. So far, many papers on the antimicrobial activity of plant extracts including essential oils have been written [28–32]. In these studies, it was stated that natural plant extracts can be used as inhibitors of fungal and bacterial pathogens, but these properties were largely determined by the plant species from which the biopreparations were obtained.

The analysis of variance shows that the plant species from which plant extracts were obtained significantly influenced the colonization of seeds of *Sinapis alba* L. by fungal pathogens. Among the analysed biopreparations, in the first term, 70% of them inhibited the development of fungi on the examined plant material (Table 1). The lowest number of infested grains, amounting to 1.0% on average, was obtained after the application of extracts from: *Coriandrum sativum* L., *Juniperus communis* L., *Carum carvi* L., *Quercus robur* L., *Arctium lappa* L., and *Calendula officinalis* L. In the second term of the study, 55% of plant species used in the analyses showed inhibitory effect on the surface development of fungi. The lowest number of infested seeds, ranging from 1.00 to 33.50%, was observed after treatment with biopreparations obtained from: *Levisticum officinale* L., *Coriandrum sativum* L., *Pinus sylvestris* L., *Satureja hortensis* L., *Lavandula vera* L., *Salix alba* L., *Ribes nigrum* L., *Artemisia absinthium* L., *Verbascum thapsiforme* L., *Juniperus communis* L. and *Carum carvi* L. Only *Hyssopus officinalis* L., *Linum usitatissimum* L., and *Arctium lappa* L. In addition, *Camelia sinensis* L. showed activity similar to the control object, while other herbal species stimulated the development of fungal pathogens on seeds of *Sinapis alba* L. On average, the number of infected seeds ranged from 62.37 (*Archangelica officinalis* L.) to even 83.50% (*Quercus robur* L.).

Antimicrobial activity of plant extracts results mainly from the presence in their composition of biologically active substances capable of inhibiting the development of microorganisms. These preparations contain mainly tannins, alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, and glycosides [27,33]. The qualitative and quantitative composition of biopreparations may vary according to the species of plant from which it was prepared. This is confirmed by Nostro et al. [34] studies, which showed that the composition of plant preparations was varied and depended mainly on the plant species. The extract prepared from *Helichrysum italicum* L. contained such ingredients as coumarins, flavonoids, steroids, and terpenes. In turn, alkaloids were present only in extracts obtained from *Nepeta cataria* and *Phytolacca dodecandra*.

No statistically significant differences were found in the method of obtaining water plant extracts for white mustard seed contamination (Table 2). However, in the first term of the evaluation both macerates and infusions did not show any inhibitory effect on fungal development in relation to control

and the number of infected seeds was 3.70% and 4.49%, respectively. The inhibitory effect was found only 7 days after the experiment was set up.

**Table 1.** Normally sprouted grains (%) depending on the species of plant from which the extracts were obtained and the method of preparation.

Herbal plant species	Degree of infestation of seeds by fungal pathogens in the first term	Degree of infestation of seeds by fungal pathogens in the second term
<i>Levisticum officinale</i> L.	1.25	17.00
<i>Coriandrum sativum</i> L.	1.00	1.00
<i>Pinus sylvestris</i> L.	1.25	5.50
<i>Satureja hortensis</i> L.	1.75	13.62
<i>Lavandula vera</i> L.	1.50	1.25
<i>Linum usitatissimum</i> L.	1.50	54.87
<i>Quercus robur</i> L.	1.00	83.50
<i>Arctium lappa</i> L.	1.00	54.25
<i>Calendula officinalis</i> L.	1.00	69.37
<i>Juglans regia</i> L.	1.87	81.37
<i>Salix alba</i> L.	3.00	24.25
<i>Origanum majorana</i> L.	21.62	69.75
<i>Archangelica officinalis</i> L.	12.62	62.37
<i>Ribes nigrum</i> L.	12.37	27.00
<i>Camelia sinensis</i> L.	10.00	53.50
<i>Artemisia absinthium</i> L.	3.50	16.12
<i>Verbascum thapsiforme</i> L.	2.25	25.25
<i>Hyssopus officinalis</i> L.	1.37	52.87
<i>Juniperus communis</i> L.	1.00	8.50
<i>Carum carvi</i> L.	1.00	33.50
Control	2.50	53.50
	LSD = 3.55%	LSD = 24.97%

**Table 2.** Influence of the method of obtaining plant extracts on limiting the development of moulds-average number of contaminated seeds (%).

Preparation of plant extract	Number of seeds infected by mould in the first term.	Number of seeds infected by mould in the second term
Macerate	3.70	40.79
Decoction	4.49	34.67
Control	2.50	53.50
	LSD = no statistically significant differences	LSD = no statistically significant differences

In the first period of the experiment, the seeds treated with macerates and infusions with fungal pathogens were characterized by the highest degree of infestation by fungal pathogens: *Origanum majorana* L., *Archangelica officinalis* L., *Ribes nigrum* L., *Camelia sinensis* L., and *Artemisia absinthium* L. On average, the level of infestation of the studied materials ranged from 6.00% to 32.75% (Table 3). After 7 days of the study, it was observed that the development of fungal microorganisms was most effectively inhibited by macerates and infusions obtained from: *Coriandrum sativum* L., *Pinus sylvestris* L., and *Lavandula vera* L. The infusions obtained from *Satureja hortensis* L., *Juniperus communis* L. and *Carum carvi* L. had similar properties and the average number of infected seeds was 1.00%. The macerates obtained from the macerates were characterized by a significant antifungal effect: *Levisticum officinale* L. and *Artemisia absinthium* L., after the application of which the presence of fungi was observed in 7.00% and 6.50% of white mustard seeds respectively.

It is assumed that the presence of flavonoids and terpenes in plant extracts and a certain degree of their lipophilicity may limit the development of moulds due to the interaction of these compounds with cell membrane components [34,35].

**Table 3.** The influence of the method of obtaining plant extracts on the degree of seed infestation by fungal pathogens (%).

Herbal plant species	Degree of infestation of seeds by fungal pathogens in the first term		Degree of infestation of seeds by fungal pathogens in the second term	
	Macerate	Decoction	Macerate	Decoction
<i>Levisticum officinale</i> L.	1.50	1.00	7.00	27.00
<i>Coriandrum sativum</i> L.	1.00	1.00	1.00	1.00
<i>Pinus sylvestris</i> L.	1.00	1.50	1.75	9.25
<i>Satureja hortensis</i> L.	1.25	2.25	26.25	1.00
<i>Lavandula vera</i> L.	1.00	2.00	1.00	1.50
<i>Linum usitatissimum</i> L.	1.50	1.50	41.50	68.25
<i>Quercus robur</i> L.	1.00	1.00	86.50	80.50
<i>Arctium lappa</i> L.	1.00	1.00	76.50	32.00
<i>Calendula officinalis</i> L.	1.00	1.00	57.00	81.75
<i>Juglans regia</i> L.	2.00	1.75	90.50	72.25
<i>Salix alba</i> L.	1.25	4.75	17.00	31.50
<i>Origanum majorana</i> L.	10.50	32.75	51.25	88.25
<i>Archangelica officinalis</i> L.	14.75	10.50	71.25	53.50
<i>Ribes nigrum</i> L.	11.50	13.25	27.25	26.75
<i>Camelia sinensis</i> L.	12.50	7.50	50.50	56.50
<i>Artemisia absinthium</i> L.	6.00	1.00	6.50	25.75
<i>Verbascum thapsiforme</i> L.	1.50	3.00	32.25	18.25
<i>Hyssopus officinalis</i> L.	1.75	1.00	88.75	17.00
<i>Juniperus communis</i> L.	1.00	1.00	16.00	1.00
<i>Carum carvi</i> L.	1.00	1.00	66.00	1.00
Control	2.50		53.50	
	LSD = 5.025%		LSD = 35.35%	

Statistical analysis showed that the herbal plant species from which biological preparations were obtained had a significant effect on the degree of infestation of *Sinapis alba* L. seeds by bacterial microorganisms. It was found that biopreparations from 40% of herbal plant species had an inhibitory effect on the development of bacteria on seeds (Table 4). This development was most effectively limited both after 3 and 7 days from the establishment of the experiment: *Quercus robur* L., *Calendula officinalis* L., *Juglans regia* L., *Salix alba* L., *Origanum majorana* L., *Archangelica officinalis* L., *Ribes nigrum* L., and *Camelia sinensis* L. Additionally, it was observed that 35% of all the examined plant species stimulated the growth of bacterial microorganisms on the surface of white mustard seeds. The highest percentage of infected seeds after 3 days by bacteria was obtained after application of extracts from: *Coriandrum sativum* L. (49.75%), *Carum carvi* L. (40.61%), *Juniperus communis* L. (34.00%), *Pinus sylvestris* L. (19.62%), *Artemisia absinthium* L. (19.50%), and *Verbascum thapsiforme* L. (15.50%).

No significant influence of the preparation of biological preparations on the level of inhibition of bacterial pathogens development on the surface of white mustard seeds was found in both study dates (Table 5).

The strongest antibacterial activity after 3 days of the study was found in infusions and macerates obtained from *Quercus robur* L., *Calendula officinalis* L., *Juglans regia* L., *Salix alba* L., *Origanum majorana* L., *Archangelica officinalis* L., *Ribes nigrum* L., and *Camelia sinensis* L. In the second term of analysis, a strong bacterial inhibitory effect was observed on the seeds that were soaked in macerate obtained from *Quercus robur* L., *Calendula officinalis* L., *Juglans regia* L., *Salix alba* L., *Archangelica officinalis* L., and *Ribes nigrum* L. In each of these cases, the number of infested seeds did not exceed 1.23% (Table 6). The infusions obtained from *Salix alba* L. and *Origanum majorana* L. where the average number of infected seeds was 1.17% and 1.10%, respectively, had similar properties. The remaining analyzed plant extracts acted at the level of the control object or significantly stimulated the development of bacteria on the surface of white mustard seeds.

**Table 4.** Influence of herbal plant species on bacterial contamination of seeds (%).

Herbal plant species	Degree of infestation of seeds by bacterial pathogens in the first term	Degree of infestation of seeds by bacterial pathogens in the second term
<i>Levisticum officinale</i> L.	3.75	41.00
<i>Coriandrum sativum</i> L.	49.75	49.75
<i>Pinus sylvestris</i> L.	19.62	96.12
<i>Satureja hortensis</i> L.	7.37	82.75
<i>Lavandula vera</i> L.	4.25	91.25
<i>Linum usitatissimum</i> L.	2.37	26.37
<i>Quercus robur</i> L.	1.37	2.25
<i>Arctium lappa</i> L.	2.87	39.50
<i>Calendula officinalis</i> L.	1.00	4.00
<i>Juglans regia</i> L.	1.00	4.75
<i>Salix alba</i> L.	1.00	1.00
<i>Origanum majorana</i> L.	1.25	2.37
<i>Archangelica officinalis</i> L.	1.25	1.87
<i>Ribes nigrum</i> L.	1.00	1.50
<i>Camelia sinensis</i> L.	1.00	3.87
<i>Artemisia absinthium</i> L.	19.50	35.12
<i>Verbascum thapsiforme</i> L.	15.50	19.00
<i>Hyssopus officinalis</i> L.	10.37	32.62
<i>Juniperus communis</i> L.	34.00	77.62
<i>Carum carvi</i> L.	40.00	52.62
Control	2.75	10.87
	LSD = 12.52%	LSD = 20.24%

**Table 5.** Influence of the method of obtaining plant extracts on limiting the development of bacteria - average number of contaminated seeds (%).

Preparation of plant extract	Number of seeds infected by mould in the first term.	Number of seeds infected by mould in the second term
Macerate	12.86	31.20
Decoction	8.97	35.34
Control	2.75	10.87
	LSD = no statistically significant differences	LSD = no statistically significant differences

A different effect of water plant extracts on the degree of contamination of seeds of *Sinapis alba* L. was found. These differences were caused mainly by the plant species from which the extracts were obtained. According to Colla et al. [36], biopreparations may differ in the composition of their active substances, which may affect different reactions in plants and seed material. These differences are mainly due to the variable sensitivity thresholds of one or more bioactive molecules [36]. It is possible that in the examined seed material, some of the analysed plant extracts contained compounds to which white mustard seeds were sensitive. This may have resulted in decreased activity of defense mechanisms and increased contamination with bacterial and fungal pathogens on seeds.

However, the search for new ways to combat microorganisms should continue, as bacteria and fungi have the genetic capacity to acquire and transfer resistance to the means used to inhibit their development [37]. The antimicrobial activity of water extracts used as seed mortars was also studied by Czerwinska et al. [38]. These studies showed that the contamination of yellow lupine and pea seeds was the most limited after application of extracts obtained from onion *Allium sativum* L., leaves of *Betula verrucosa* L. and roots of *Levisticum officinale* L. On the other hand, studies conducted by Szparaga et al. [39] determining the vitality and healthiness of seeds of *Brassica oleracea* L. showed that natural extracts obtained from *Carum carvi* L., *Archangelica officinalis* L. and *Salix alba* L. improved the healthiness of the analysed materials to the greatest extent. The obtained values of control deviations obtained by the authors ranged from  $-69.03\%$  to  $-78.4\%$ . In our own research, also extracts obtained from *Archangelica officinalis* L. significantly inhibited the surface development of fungi.

**Table 6.** The influence of the method of obtaining plant extracts on the degree of seed contamination by bacterial microorganisms (%).

Herbal plant species	Degree of infestation of seeds by bacterial pathogens in the first term		Degree of infestation of seeds by bacterial pathogens in the second term	
	Macerate	Decoction	Macerate	Decoction
<i>Levisticum officinale</i> L.	3.50	3.25	53.25	28.75
<i>Coriandrum sativum</i> L.	39.25	21.28	59.25	40.25
<i>Pinus sylvestris</i> L.	23.50	15.75	99.50	92.75
<i>Satureja hortensis</i> L.	6.25	8.50	72.75	92.75
<i>Lavandula vera</i> L.	3.50	5.00	89.75	92.75
<i>Linum usitatissimum</i> L.	3.00	1.75	45.75	7.10
<i>Quercus robur</i> L.	1.75	1.10	1.00	3.51
<i>Arctium lappa</i> L.	4.75	1.00	23.75	55.25
<i>Calendula officinalis</i> L.	1.00	1.00	1.11	7.00
<i>Juglans regia</i> L.	1.00	1.12	1.00	8.50
<i>Salix alba</i> L.	1.40	1.00	1.00	1.17
<i>Origanum majorana</i> L.	1.51	1.08	3.75	1.10
<i>Archangelica officinalis</i> L.	1.09	1.25	1.23	2.75
<i>Ribes nigrum</i> L.	1.00	1.00	1.14	2.00
<i>Camelia sinensis</i> L.	1.20	1.21	5.50	2.25
<i>Artemisia absinthium</i> L.	37.50	19.50	51.75	24.50
<i>Verbascum thapsiforme</i> L.	29.50	15.50	41.25	22.75
<i>Hyssopus officinalis</i> L.	2.75	10.37	1.00	64.25
<i>Juniperus communis</i> L.	3.25	34.07	68.00	87.25
<i>Carum carvi</i> L.	41.25	40.62	58.75	89.50
Control		2.75		10.87
		LSD = 4.93%		LSD = 28.63%

#### 4. Conclusions

The obtained results showed that the activity of extracts in limiting the contamination of white mustard seeds by microorganisms depended mainly on the plant species from which they were obtained.

The most effective extracts inhibiting fungal growth on the seed surface were the preparations obtained from *Levisticum officinale* L., *Coriandrum sativum* L., *Pinus sylvestris* L., *Satureja hortensis* L., *Lavandula vera* L., and *Juniperus communis* L. However, the greatest reduction in bacterial contamination of the examined seeds was obtained in combinations where preparations from *Quercus robur* L. were used as mortar, *Calendula officinalis* L., *Juglans regia* L., and *Ribes nigrum* L. Additionally, the studies showed that both the macerate and the infusion obtained from *Salix alba* L. had the most favourable effect on microbiological purity of the seeds of *Sinapis alba* L.

In view of the ever-increasing consumer awareness of safe food production methods, it is necessary to seek out techniques to meet these expectations. One such technique, as shown by research, may be the treatment of seeds with natural preparations that are safe for the environment.

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