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The potential of volatiles from *Brassica juncea* seeds against grey mould agent *Botrytis cinerea* and their effect on storage and sensory quality of spinach leaves

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Abstract: The potential use of volatile compounds released from milled seeds of mustard (*Brassica juncea* cv. Malopolska) obtained from three different companies was tested in *in vitro* and *in vivo* experiments for their inhibitory effect on *Botrytis cinerea* growth on agar media and its infection on vegetable leaves of cucumber, bean and spinach. In the experiments with spinach, the effect of volatiles from mustards on the storage and sensory quality of fumigated leaves was evaluated. The antifungal effect of the volatiles depended on the source and dosage of mustard seeds and biofumigation time. The most efficient inhibition of B. cinerea mycelium growth on agar media and vegetable leaves was mustard S from SHR company. The development of grey mould on spinach leaves was inhibited in the treatment with 4 h biofumigation with the volatiles from mustard S seeds in experiments conducted at 10 °C and also at 18 °C. In the sensory and storage quality analysis, the spinach leaves treated with volatiles from mustard seeds showed acceptable parameters that predisposed the product to consumption. The results show that it is possible to reduce the incidence of vegetable grey mould with the treatment of milled mustard seeds, opening a potential application of biofumigation in the control of *B. cinerea* in vegetables.

Keywords: biofumigation; mustard; grey mould; cucumber; bean

Botrytis cinerea Pers. Ex. Fr. is one of the most extensively studied necrotrophic fungal pathogens and causes grey mould rot in more than 500 plant species. This pathogen has a disastrous economic impact on various economically important crops, including vegetables (e.g. lettuce, spinach, bean, tomato) and fruit (e.g. grape, strawberry, blueberry), and it can be present inside stems, leaves, flowers, fruits and seeds (Aktaruzzaman et al. 2017; Garfinkel 2021). Importantly, *B. cinerea* continues to attack new plant species and is identified as a pathogen in new environments (Azevedo et al. 2020; Ally et al. 2021; Ismagulova et al. 2021). Due to its scientific and economic importance, *B. cinerea* has been classified as an "important plant pathogen". *B. cinerea* also causes secondary soft rots of fruit and vegetables in storage, transit, and market. Post-harvest decays of vegetables and fruits account for significant levels of post-harvest losses. About 20–25% of fruits and vegetables are estimated to be

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decayed by pathogens during post-harvest handling in developed countries. Moreover, in developing countries, post-harvest losses are often more severe due to inadequate storage and transportation facilities (Garfinkel 2021; Bi et al. 2023).

It is difficult to control *B. cinerea* because it has a broad host range of various attack modes, and both asexual and sexual stages can survive in favourable or unfavourable conditions (Hua et al. 2018). The following strategies have been used to control *Botrytis*-incited diseases: chemical control, resistance inducer, and biological control. Applying synthetic fungicides to control diseases caused by this pathogen is the main method in crop production. However, the protective effects of fungicides are not satisfactory on *B. cinerea*, whose genome is plastic and prone to developing drug-resistance genes (Shao et al. 2021). In addition, fungicides are not safe for humans and the environment; they are costly and cause biodiversity loss (Rani et al. 2021; Pathak et al. 2022). Therefore, finding new environmentally safe methods to reduce *B. cinerea* is important, especially during the shelf-life of vegetables and fruits. A promising way to protect against *B. cinerea* is using biologically active substances obtained from plants. Plants are a rich source of secondary metabolites – characterised by biological activity. The vast majority of higher plants, as many as 85%, secrete compounds into the surrounding environment that affect living organisms. Among numerous biologically active plant compounds such as phenols, aldehydes, alcohols, essential oils, acids and terpenes, many have inhibitory or lethal effects on bacterial and fungal plant pathogens (Aguilar-Gonzalez et al. 2015; Redondo-Blanco et al. 2020; Chen et al. 2023).

Among them, mustard *Brassicaceae* plants are of special interest for biofumigation use. Plants belonging to this family contain significant levels of glucosinolate compounds. About 20 different types of glucosinolates are commonly found in *Brassicaceae* (Lietzow 2021). During plant tissue destruction, glucosinolates are hydrolysed by myrosinase enzymes in the presence of water. Myrosinase is stored in specialised myrosin cells located separately within the plant body. When the tissues are damaged by mechanical stress or microbial degradation, myrosinase decomposes glucosinolate, forming many products, including isothiocyanates, thiocyanates, nitriles, and oxazolidinethiones. Isothiocyanates are the most toxic of several hydrolysis products and are known to have broad biocidal activity (Sikorska-Zimny & Beneduce 2020). The compound is also present in mustard plants (*Brassica juncea)*. Mustard, especially the seeds, is commonly used for food seasoning or as a medicinal plant.

Some studies reveal that volatile compounds released from milled seeds of mustard inhibit the growth of certain plant pathogenic microorganisms. For example, Kowalska and Smolińska (2008) proved that milled seeds of mustard used as a source of volatile compounds, inhibited soft rot of onion bulbs caused by *Pectobacterium carotovorum* subsp. *carotovorum*, *Burkholderia cepacia* and *Burkholderia gladioli* pv. *alliicola*. Many literature reports indicate the antifungal activity of glucosinolates against *Verticillium dahliae, Rhizoctonia solani, Fusarium oxysporum* (Poveda et al. 2020; Plaszko et al. 2021; Ziedan 2022). Ugolini et al. (2014) found that ally-isothiocyanate, the compound present in seeds of mustard, inhibited the growth of *B. cinerea in vitro* and *in vivo* trials reduced the decay of strawberries.

In the context of the present study, it is important to note that the content of glucosinolates in *Brassicaceae* plants depends not only on the plant variety and genetic background of plants but also on the plant organs. It is known that the content of active agents in the seeds is much higher than in the leaves, stems or roots. Furthermore, it is reported that the glucosinolate content in the same species and cultivar and the same plant parts is influenced by environmental factors such as temperature, fertility, fertilisers, and biological or chemical protection agents during plant growth. Storage conditions of plant materials also can change the content of glucosinalates (Sikorska-Zimny & Beneduce 2020; Tian & Deng 2020; Sikorska et al. 2023; Zhu et al. 2023).

The objective of this study was to investigate the effect of volatile compounds released from milled seeds mustard obtained from three different companies on *(i) B. cinerea* growth and development of its sclerotia on microbiological media; *(ii)* the development of grey mould on bean, cucumber and spinach leaves; *(iii)* the effect on the sensory and storage quality of spinach leaves.

MATERIAL AND METHODS

The experiments used the strain of *B. cinerea* from pathogenic fungi collection in a microbiological laboratory. The strain was originally isolated from

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broccoli, and their pathogenicity was confirmed. Pathogenicity tests were conducted by artificial inoculation of some main vegetable species, including cucumber, bean and spinach plants. The strain spore suspension (density 7–9 × 106 mL^{-1}) was inoculated on growing plants in pots. The disease symptoms characteristic of grey mould were observed after 10–14 days. The strain was stored on the PDA (potato dextrose agar, Merck) medium at 4–8 °C until use. The *B. cinerea* sclerotia were produced on PDA medium for 10 days at 24 °C. Seeds of mustard (*Brassica juncea*) cv. Malopolska were obtained from three different companies in Poland: SHR (seeds S), Kampol (seeds K), and Piast (seeds P). The seeds were stored at 8 °C in the laboratory. Just before use, the seeds were milled in the electric mill. In the laboratory, the concentration of allyl isothiocyanate in milled and wetted mustard seeds was measured according to the method with $AgNO_3$ described by Abul-Fadl et al. (2011). The seeds S, K, and P contained 272.72, 94.40 and 36.30 mg in 100 g dry mass, respectively.

The effect of fumigation with milled mustard seeds on mycelial growth of *B. cinerea* on agar medium. The experiment was conducted in big glass Petri plates (diameter 18 cm, volume 314 cm^3) used as incubation chambers. Five smaller Petri plates (45 mm diameter) filled with PDA medium were situated on each plate. The small plates were inoculated in the centre with *B. cinerea* mycelial, and their lids were removed. In the middle of the big incubation plate, among fungus inoculated plates, one additional Petri plate (45 mm) was put, which contained milled seeds of mustard S, K or P at doses 0.25 g, 0.5 g or 1 g. The mustard powder was watered with 0.25 mL, 0.5 mL, and 1 mL of distilled water. The plate with 1 ml of water was used in the control treatment. Immediately after mustard wetting, each incubation chamber was sealed with parafilm and put into the incubator at a temperature set at 25 °C. After 1 h or 3 h of fumigation, the central Petri plates with mustard powder were removed. The incubation chambers were sealed again, and the incubation lasted for 4 days. Then, a mycelial diameter of *B. cinerea* was measured.

The effect of fumigation with milled mustard seeds on *B. cinerea* sclerotia germination. The experiment was performed as described above, but instead of *B. cinerea* mycelium inoculation PDA medium in small plates, sclerotia of this pathogen were used. Each plate was inoculated in the centre with one sclerotium. The doses of mustard powder in central plates were increased in this test because 0.25 g and 0.5 g appeared ineffective in the previous experiment. The doses of mustard S, K and P were 0.75 g, 1 g and 2 g. The powders were wetted with 0.75 mL, 1 mL and 2 mL of distilled water, respectively. After sealing, the incubation chambers were put into the incubator with a temperature set at 25 °C. After 3 h of fumigation, the central Petri plates with mustard powder were removed. The incubation chambers were sealed again, and the incubation lasted 6 days. During incubation, germination of sclerotia was noted, and the diameter of mycelium growing from them was measured after 2, 3, 4 and 6 days.

The impact of volatile compounds from mustard seeds on grey mould on vegetable leaves. The detached leaves of bean (*Phaseolus vulgaris* L.) cv. Ibiza, cucumber (*Cucumis* L.) cv. Iva and spinach (*Spinacia oleracea* L.) cv. Rembrandt was used *in vitro* experiments in big glass Petri plates (diameter 18 cm, volume 314 cm^3). Bean and cucumber's second fully developed leaves were collected from plants grown in a growth chamber (Sanyo) in multi-pots. Fresh spinach leaves were obtained from a commercial, horticultural farm (Wróblewo, Poland). The leaves were placed on water-moistened blotting paper on the plates: five leaves for bean and cucumber and seven for spinach.

The leaves were gently damaged with a glass rod in their central part and inoculated in this spot with 20 μL of the spore suspension of *B. cinerea* at the density 7–9 × 106 mL^{-1}, prepared from a 3-week-old culture of *B. cinerea* on PDA medium. The spore density was measured using a hemacytometer under a microscope (Olympus BX 41).

In the tests with bean and cucumber, a small glass petri plate (45 mm) containing 5 g of milled seeds of mustard S was placed in the centre of the large plate among inoculated leaves. Then, the mustard powder was moistened with 5 mL of water, and the plate was immediately sealed with parafilm. In the tests with spinach, two doses of mustard milled seeds S and P were used: 0.75 g and 1 g, and moistened with 0.75 or 1 mL of water, respectively. The spinach leaves are more tender and sensitive; therefore, the doses of mustard powder were reduced. In the control treatments in all tests, the plates with water were put among the leaves instead of the small plates with mustard powder.

The sealed plates with bean and cucumber leaves were incubated for 8 days under provocative con-

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ditions at 25 °C. Necrosis diameter on each bean and cucumber leaf was measured at 2, 4, 6 and 8 days of incubation. In the tests with spinach, the plates with mustard powder were removed after 2 or 4 h of fumigation. Then, the leaves were incubated at 18 °C in the dark for consecutive 4 days as a simulation of conditions during supermarket sales. The diameter of *Botrytis* caused necrosis on the leaves was measured on the 4th day.

The experiments described above were conducted three times, and each treatment was prepared in three or four replications.

The impact of volatile compounds from mustard seeds on grey mould development, sensory and storage features of spinach. As spinach leaves are often stored fresh before consumption, it was decided to test their storage quality. On the other hand, taste is also an important aspect, which volatile compounds from mustard can influence. Therefore, the sensory quality of the spinach after biofumigation was checked.

The experiment was carried out in airtight plastic containers of 3 000 cm^3. In each container, 100 g of freshly harvested spinach leaves were placed. In three places, a batch of the leaves in the container was inoculated with a fragment of *B. cinerea* mycelium. Then, a small plate (45 mm) containing 8.5 g of milled mustard seeds, S or P, was placed in a container. The containers were closed tightly and placed in a cold store at 10 °C. After 2 or 4 h, the plates with mustard powder were removed from the containers, and the leaves were stored in the same condition for 20 days. There were the following treatments: *(i)* control without mustard; *(ii)* 2 h fumigation with S; *(iii)* 4 h fumigation with S; *(iv)* 2 h fumigation with P; *(v)* 4 h fumigation with P. In all treatments, the leaves were inoculated with *B. cinerea*. The same treatments but with spinach leaves not inoculated with *Botrytis* were prepared for sensory analysis. Each treatment was prepared in three replications. The leaves were systematically monitored during storage to assess the development of grey mould symptoms. After 12, 14, and 20 days, the diameter of the lesions on the leaf surface was measured.

The sensory examination of treated with mustard S and P spinach leaves was performed two times: immediately after 2 and 4 h fumigation and 24 h after fumigation. The method of Quantitative Description Analysis, i.e., sensory profiling, was used in accordance with ISO (2016). The assessment was carried out in the sensory laboratory, meeting the requirements of the standard PN-ISO 8589 (2007). A 10-person team of experts performed the evaluation. During the analysis, each person was in the individual evaluation box equipped with a computer and specialised software (ANALSENS version 7) designed to prepare tests, record individual assessments and process the results. For sensory evaluation of spinach leaves, the following quality descriptors were selected: spinach smell, off-smell, colour, firmness, spinach flavour, bitter taste, off-taste, overall quality and product acceptability. The intensity of each descriptor was assessed on a graphical scale, corresponding to 0 (low intensity) – 10 (high intensity) conventional units, with marginal markings. The evaluation was carried out in two sessions.

Leaves of spinach biofumigated with S mustard seeds were prepared for quality assessment. Spinach leaves (60 g) were placed into a plastic container with a volume of 1 750 cm^3. The following treatments were studied: control (not fumigated leaves), 2 h-fumigation, and 4 h-fumigation. The dosage of milled mustard seeds for fumigation was 5 g per container. The experiment was conducted in a storage room at 10 °C. Quality assessment was performed after 3 and 6 days of storage. The following vegetable characteristics were visually assessed: wilting/softening, browning/discoloration of the cut surface, rotting and commercial value. The evaluation was conducted based on the 9-grade scoring scale presented in Table 1. Weight loss was also determined, which was expressed as percentage weight differences from the initial weight. The experiment was conducted in four replications and it was repeated.

Statistical analyses. The results were statistically analysed using a one-way analysis of variance with the Tukey test, $P < 0.05$, using the statistical program Statistica (version 13.1). Data not significantly different from each other are marked with the same letters. Sensory characteristics of the evaluated plant material were described using principal component analysis (PCA) based on a correlation matrix. Calculations were carried out in the statistical package Statistica (version 13.1).

RESULTS

The effect of fumigation using milled mustard seeds on the growth of *B. cinerea* on the agar

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Table 1. The influence of fumigation with mustard S on spinach storage quality

Treatment	Wilting	Discolouration	Rotting	Marketable value	Weight loss (%)
3 days of storage					
Control	2.88 ± 0.48	3.13 ± 0.25	1.00 ± 0.00	6.75 ± 0.65	16.6 ± 7.75
2 h fumigation	3.50 ± 0.91	3.25 ± 0.50	1.00 ± 0.00	5.88 ± 0.85	18.7 ± 2.52
4 h fumigation	3.63 ± 0.25	3.63 ± 0.95	1.00 ± 0.00	5.75 ± 0.50	24.3 ± 3.17
6 days of storage					
Control	4.13 ± 0.63	3.88 ± 0.25	1.00 ± 0.00	4.50 ± 0.82	23.85 ± 5.37
2 h fumigation	4.38 ± 0.48	4.50 ± 0.41	1.00 ± 0.00	3.88 ± 0.63	26.50 ± 3.30
4 h fumigation	3.88 ± 0.25	4.00 ± 0.91	1.00 ± 0.00	4.75 ± 0.65	31.3 ± 2.68

Averages from four replications ± s.d.; the data were not statistically different at $P < 0.05$ by ANOVA and Tukey test; wilting: 1 – no wilting and softening, 3 – light, 5 – medium, 7 – strong, 9 – very strong; discolouration: 1 – no discolouration, 3 – very beginning of discolouration, 5 – light brown, 7 – medium brown, 9 – brown; rotting: 1 – no rotting, 3 – up to three small spots, 5 – quite strong, 7 – strong, 9 – very strong; marketable value: 1 – no marketable value, 3 – limited, 5 – fair, 7 – good, 9 – excellent

medium. The antifungal effect of the volatiles released from milled mustard seeds was highly related to the dose of the seed powder, fumigation time as well as the origin of a seed (Table 2). One-hour fumigation by all tested seeds was completely inefficient (data not presented). The most inhibitory effect was observed with S mustard seeds when fumigation time was prolonged to 3 h. Mustard seeds S used at the 1g dose completely inhibited the growth of *B. cinerea* mycelium. Lower doses of the mustard –0.25 g and 0.50 g, were less effective. However, mycelial growth was markedly reduced in both cases compared to the control (28.5 mm and 28.2 mm diameter, respectively). For P and K mustard, significant inhibition of *B. cinerea* growth was observed when fumigation lasted 3 h and only at the highest doses of milled seeds (Figure 1A). Reducing the dose to 0.50 g and 0.25 g at 3 h fumigation gave a limited inhibitory effect, but the differences were not significant compared to control (Table 2).

Table 2. The influence of volatiles released from milled seeds of mustard on the growth of *Botrytis cinerea* mycelium

Mustard dose (g)	*Botrytis cinerea* mycelium diameter (mm) – 3 h fumigation		
	Mustard S	Mustard P	Mustard K
0.00	45.0^{c}	45.0^{c}	45.0^{c}
0.25	28.5^{b}	38.4^{c}	42.6^{c}
0.50	28.2^{b}	35.2^{c}	40.2^{c}
1.00	0.0^{a}	15.2^{b}	18.6^{b}

Similar letters above the means indicate a significant difference at $P < 0.05$ by ANOVA and Tukey's test

One-hour biofumigation proved to be completely ineffective regardless of the dose of mustard used (data not presented).

In the next experiment, the fumigant effect of milled mustard seeds on *Botrytis* sclerotia germination and subsequent mycelium growth was studied. A 3 h fumigation time was implemented. After 2 days of incubation, sclerotia germination was observed only in the control group with no fumigated sclerotia. In all combinations with mustard, germination of sclerotia was completely inhibited, irrespective of the dose and type of mustard (Table 3). After 3 days of incubation, growth of the sclerotia treated with mustard S was still completely inhibited, but in the combinations with mustard P and mustard K, *B. cinerea* started to grow. In the case of mustard P the growth was more delayed and slower than in mustard K and control treatments. On the next incubation day (day 4), sclerotia fumigated with mustard S and started to germinate, but the growth of mycelium was still significantly inhibited compared to other mustards. However, after 6 days, intensive growth also occurred in mustard S treatment. The most effective delaying effect on sclerotia germination and mycelium growth was observed for mustard S, followed by mustard P, and the least effective was mustard K (Table 3).

The impact of volatile compounds released from mustard seeds on grey mould on vegetable leaves. The experiments with bio-fumigated, detached leaves of beans, cucumber and spinach showed a strong impact of volatile compounds released from milled mustard seeds. The leaves were

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Table 3. The effect of volatiles released from milled seeds of mustard on germination of *Botrytis cinerea* sclerotia and subsequent mycelium growth on PDA medium

Mustard dose (g)	Mycelium diameter (mm) after incubation for days			
	2	3	4	6
Control 0	27.7^{b}	45.0^{e}	45.0^{b}	45.0^{a}
Mustard S				
0.75	0.0^{a}	0.0^{a}	10.9^{a}	38.0^{a}
1.00	0.0^{a}	0.0^{a}	12.0^{a}	38.3^{a}
2.00	0.0^{a}	0.0^{a}	10.0^{a}	41.7^{a}
Mustard P				
0.75	0.0^{a}	3.8^{ab}	40.2^{b}	45.0^{a}
1.00	0.0^{a}	5.3^{abc}	45.0^{b}	45.0^{a}
2.00	0.0^{a}	1.3^{a}	32.8^{b}	45.0^{a}
Mustard K				
0.75	0.0^{a}	19.3^{d}	45.0^{b}	45.0^{a}
1.00	0.0^{a}	14.1^{cd}	45.0^{b}	45.0^{a}
2.00	0.0^{a}	10.9^{bcd}	45.0^{b}	45.0^{a}

Similar letters above the means indicate a significant difference at $P < 0.05$ by ANOVA and Tukey's test

inoculated with *B. cinerea* propagules. In the tests with beans and cucumbers, gradual development of necrotic lesions on plant tissues was observed only in control treatments. No growth of the fungus and no development of grey mould was observed in treatments with mustard S (Table 4, Figures 1B–1C).

Grey mould symptoms have progressed more slowly on spinach leaves than on other testing plants. The average necrosis diameter, measured after 4 days of incubation, was 4.4 mm in the control treatment. The volatiles released from mustard S completely inhibited the necrosis development on the spinach leaves, regardless of fumigation time. The spinach leaves were also treated with the volatiles released from seed P, but their effect was lower compared to seed S. In the mustard P treatment, the antifungal efficacy significantly depended on the time of biofumigation. The development of *B cinerea* was completely reduced in 4 h fumigation. In contrast, after 2 h fumigation, grey mould symptoms appeared and were dose-dependent – at 1 g dose, the diameter was 1.6 mm, while at 0.75 g dose – 3.6 mm (data not presented).

In experiments with spinach leaves inoculated with *B. cinerea* and preserved for 20 days at storage conditions, a significant reduction of grey mould

Table 4. The impact of volatile compounds from milled seeds of mustard S on the development of grey mould on leaves of cucumber and bean

Treatment	Diameter of *Botrytis cinerea* necrosis (mm) on the leaves measured after incubation for days			
	2	4	6	8
Bean				
B. cinerea	7.5^{b}	12.3^{b}	47.5^{b}	60.0^{b}
B. cinerea + mustard S	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Cucumber				
B. cinerea	3.1^{b}	20.1^{b}	40.0^{b}	60.0^{b}
B. cinerea + mustard S	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}

Similar letters above the means in the same column separately for each vegetable indicate significant difference at $P < 0.05$ by ANOVA and Tukey's test

development by mustard S was observed (Table 5). The protective effect was related to fumigation time. 2 h fumigation with mustard S reduced necrosis by approximately 24% compared to control, but this effect was insignificant. When fumigation was prolonged to 4 h, the reductive effect of mustard S volatiles significantly increased – after two weeks of storage, the mean lesion diameter on fumigated plant material was 4.9 mm, but in control, it developed to 21.9 mm. After three weeks, the necrosis reached 13.3 and 32.8 mm, respectively. The effect of volatiles from P seeds was insignificant compared to the control. However, the tendency to inhibit grey mould on spinach leaves fumigated for 4 h was observed (Table 5).

Storage and sensory quality of spinach after biofumigation. Healthy spinach leaves fumigated only

Table 5. The influence of volatiles from milled seeds of mustard S and P on the grey mould development on spinach leaves during storage at 10 °C

Treatment	Diameter (mm) measured after storage for days		
	12	14	20
Control	16.1^{b}	21.9^{b}	32.8^{b}
Mustard S for 2 h	12.8^{b}	15.6^{b}	25.0^{ab}
Mustard S for 4 h	2.1^{a}	4.9^{a}	13.3^{a}
Mustard P for 2 h	12.2^{ab}	18.2^{b}	27.8^{b}
Mustard P for 4 h	12.4^{b}	12.8^{ab}	31.1^{b}

Similar letters above the means in the same column separately for each vegetable indicate significant difference at $P < 0.05$ by ANOVA and Tukey's test

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with mustard S were chosen to study sensory and storage quality. The studied parameters of fumigated spinach showed that its quality did not differ significantly compared to control plant material (Table 1). However, after 3 days of storage, the marketable value was lower because of higher wilting and discoloration in biofumigation treatments than in control. This trend was maintained after 6 days of storage but only for a 2 h biofumigation treatment. In the case of spinach fumigated for 4 h, its storage quality was comparable to that of the control.

Statistical similarities and differences in the sensory quality of the assessed spinach leaves, treated with volatile compounds from mustard seed, are shown in Figure 1. The space on the map was determined by the first two principal components, explaining 54.6% and 13.22% of the overall variation, respectively. Overall quality was positively related

(A)
(B)
(C)

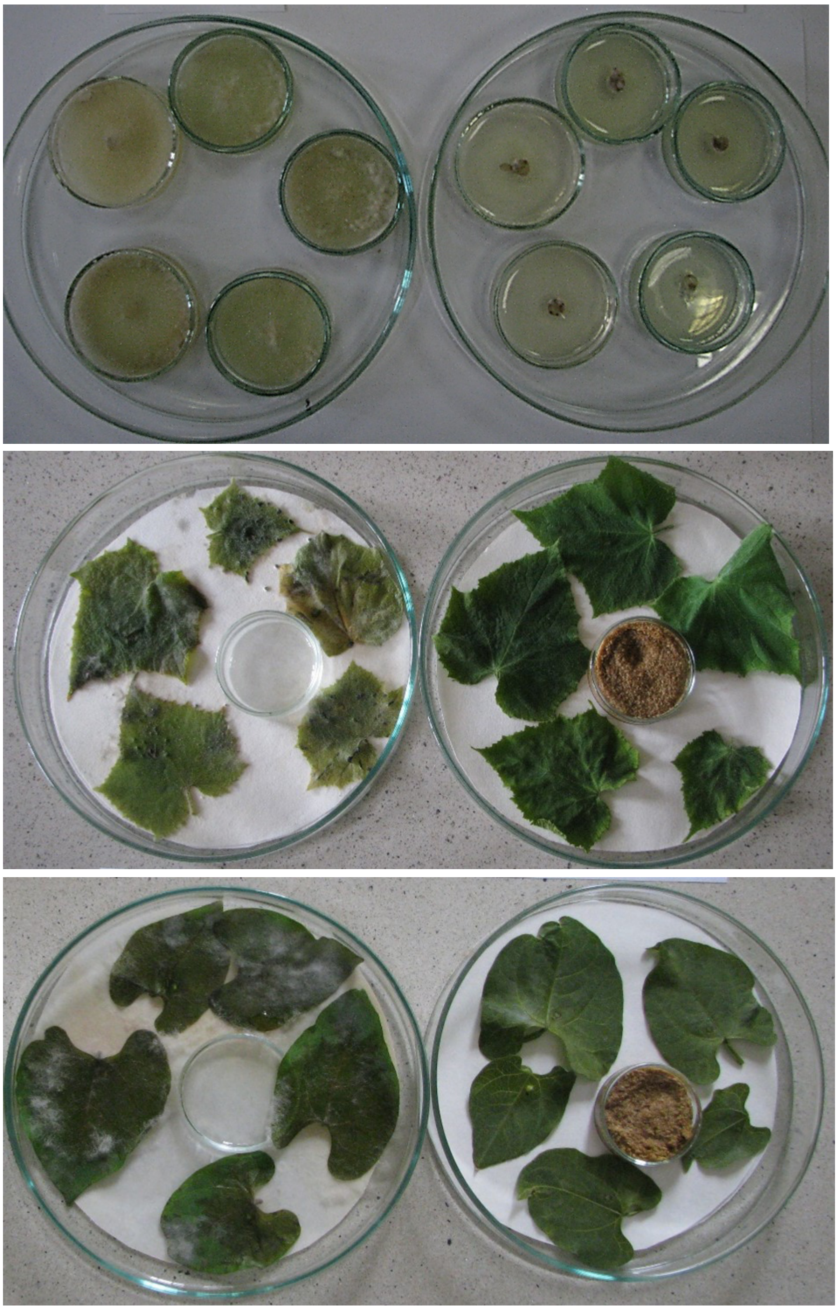

Figure 1. Sample photos of experiments in Petri plates – effect of volatiles from mustard seeds on the development of *Botrytis cinerea* on PDA medium; (A), cucumber leaves (B) and bean leaves (C); left side – control; right side – treated with mustard volatiles

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to product acceptability and firmness (vectors following the same direction). The objects treated with mustard S and P were evaluated 24 h after biofumigation, and they had the highest sensory quality and the best product acceptability (as evidenced by the close location of these treatments near these vectors). The location of the objects, mustard S (2h) and mustard P (2h), assessed immediately after biofumigation, on the opposite side of the overall quality assessment vector but close to the vectors of odour and extraneous taste, indicates a lower sensory quality of these objects compared to the others. The results may suggest that the time after biofumigation with volatile substances from mustard seeds has a greater influence on the sensory quality of spinach leaves than the source of the mustard seeds.

DISCUSSION

There is a global trend towards finding alternative methods to chemical treatments for managing plant diseases in primary production and during storage, post-harvest processing, and selling to avoid discarding plant products. It is especially important for polyphagous pathogens, causing considerable damage to numerous crops, which develop resistance to the range of fungicides, as in *Botrytis* (Shao et al. 2021). In recent years, the research for new techniques and new strategies for plant disease control led to the development of studies with biologically active plant substances (Wood et al. 2013; Aguilar-Gonzalez et al. 2015; Redondo-Blanco et al. 2020). Plants synthesise a range of compounds that can be used, but increasing attention is given to glucosinolates, secondary metabolites of *Brassicaceae* plants, and their enzymatic derivatives (Dai & Lim 2014; Poveda et al. 2020; Dubey et al. 2021; Fontana et al. 2021; Lietzow 2021). The volatile compounds released from these plants after glucosinolates hydrolysis may inhibit bacterial and fungal pathogens (Ugolini et al. 2014; Beck & Marsh 2015; Bahmid et al. 2020; Plaszko et al. 2021). However, there is less data about the activity

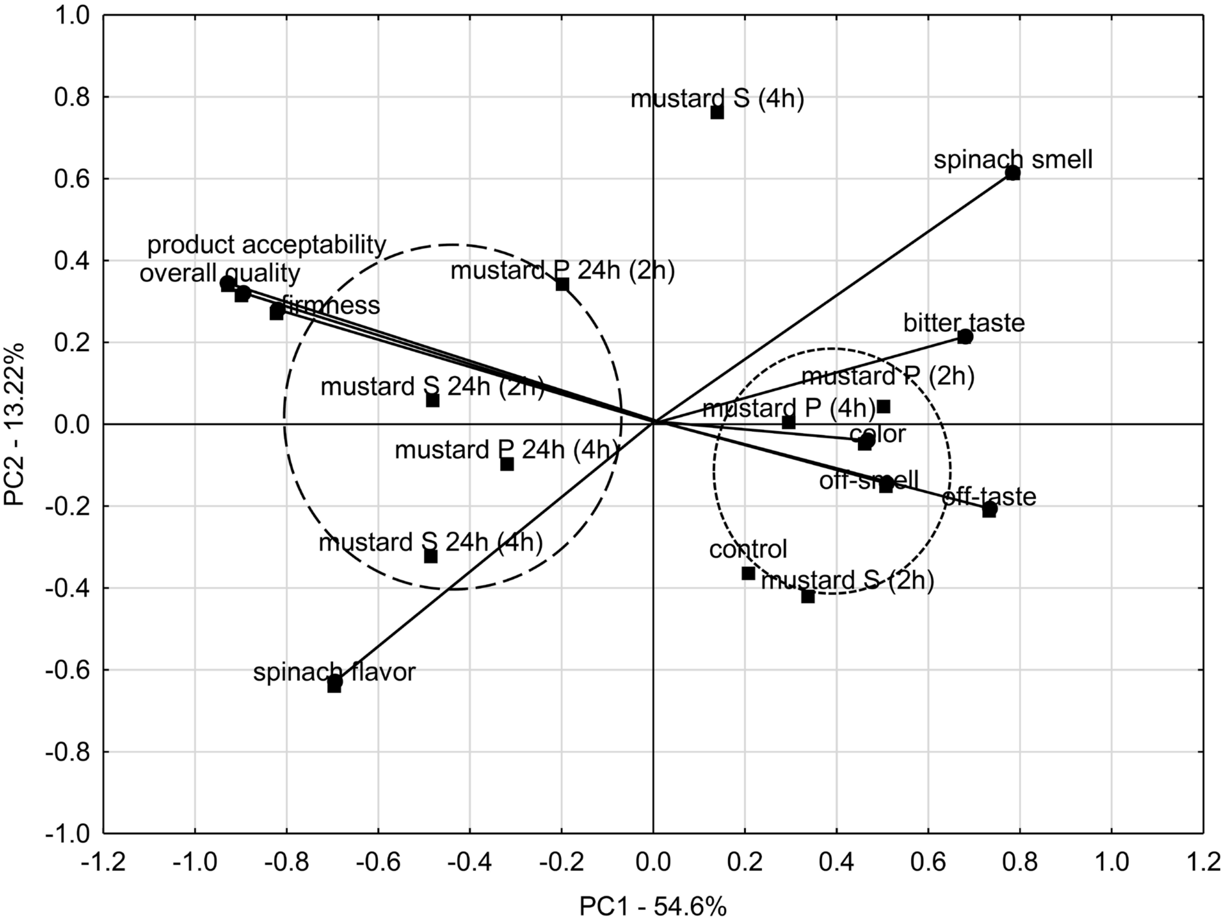

Figure 1. The influence of fumigation with mustard S and P on spinach sensory quality. Graph of the configuration of points representing variables in the first two factorial axes (principal components) system

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(2023) screened mustard cultivars to evaluate their biofumigation effect against *B. cinerea*. Among 90 screened cultivars, only one Dilong-1 completely inhibited mycelial growth and sporulation of the pathogenic fungus. Furthermore, biofumigation of apple fruits with this mustard cultivar significantly reduced grey mould.

The presented study also showed the antifungal effect of volatile compounds released from mustard seeds on the growth of *B. cinerea* mycelium and sclerotia. However, while Tian et al. (2023) have shown significant differences in fumigation activity between mustard cultivars, we showed the differences between the activity of volatile compounds released from mustard seeds of the same cultivar but obtained from different sources. The best inhibitory effect was obtained with milled seeds S of *B. juncea*, obtained from SHR company, and then with P and K seeds obtained from Kampol and Piast company, respectively. Restriction capacity of *B. cinerea* growth was positively related to the dose of milled seeds and fumigation duration.

In both Tian et al. (2023) and our studies, the inhibitory efficacy was related to the concentration of active compounds in plant material used for biofumigation. In the seeds of mustard allyl isothiocyanate, which is the active compound. Despite the same variety – Malopolska's concentration of allyl isothiocyanate differed. The seeds of mustard S, which have proved to be the most active among others used in the experiments, contained the highest amount of allyl isothiocyanate –272.72 mg in 100 g of dry seed mass. Meanwhile, mustard K and P contained 94.40 and 36.30 mg of allyl isothiocyanate in 100 g of dry seed mass. This shows that for practical use, it is essential to examine the content of the active substances in the batches of material which are to be used for biofumigation. It is not enough to use a specific variety or species of *Brassicaceae* plant, which is effective in disease suppression. It is important that the content and profile of active compounds in plant material may be dependent on many factors such as crop genetic features, cultivar, cultivation type and conditions, temperature, humidity, light intensity, water supply, drought stress, harvesting date, weather conditions during cultivation or seed storage methods (Bohnic & Trdan 2012; Neugart et al. 2018; Ben Ammar et al. 2023; Sikorska et al. 2023).

It is worth paying particular attention to the effectiveness of the tested materials in controlling sclerotia. These forms are much more difficult to destroy than mycelium. The results obtained in inhibiting the growth of sclerotia testify to the high potential of the tested material in combating plant pathogens. Yang et al. (2021) found that spore germination and fungal growth of *Aspergillus ochraceus*, *A. carbonarius* and *A. niger* were significantly inhibited by allyl isothiocyanate. Moreover, the compound reduced the infection of these fungi on grapes.

The study showed the antifungal effect of volatile compounds released from mustard seeds on the growth of *B. cinerea* mycelium and sclerotia on microbiological media and plant leaves. Our studies are in agreement with the results obtained by other researchers. The study by Aguilar-Gonzalez (2015) also demonstrated the effectiveness of vapours of mustard essential oils against *B. cinerea*. In the presented studies, *B. cinerea* growth and grey mould development were inhibited in bean, cucumber, and spinach leaves by volatiles from mustard seeds. The antifungal effect was the most efficient on bean and cucumber leaves during continuous biofumigation with mustard seeds. The literature shows some evidence of using volatiles in plant protection. Ugolini et al. (2014) exposed strawberry fruit for 4 h in an atmosphere enriched by allyl isothiocyanate or seed meals. The allyl isothiocyanate, both synthetic and released from mustard seeds, reduced the decay caused by the pathogen by over 47.4 up to 91.5%, significantly different from untreated fruit. Similar results were obtained with benzyl isothiocyanate to control grey mould rot in inoculated strawberries (Sun et al. 2021). *B. cinerea* development was also inhibited by allyl isothiocyanate released from black mustard seeds in tomatoes (Barea-Ramos et al. 2024).

An important aspect of the research was to show that spinach treated with mustard seed volatiles retains its sensory and storage qualities. These properties are very important for consumers and influence their purchasing choices. This is particularly important because spinach is often eaten raw and not heat-treated. Therefore, its quality should be high (Jung et al. 2012).

CONCLUSION

In conclusion, our results show it is possible to reduce the incidence of grey mould on vegetables with a treatment of volatile compounds re-

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leased from mustard seeds, opening the potential application of biofumigation in the control of *B. cinerea* in vegetables, especially during their storage. These results suggest that milled seeds of mustard can be used as natural fungicides to prevent *B. cinerea* from infecting spinach, cucumber, and beans. Spinach, bean, or cucumber leaves infected by *B. cinerea* in the field support latent infections that can spread during the post-harvest phase, causing a high incidence of grey mould on cucumber and bean fruit and spinach leaves during storage. Further research is needed to see if the volatile compounds from mustard seeds also work for other spinach, cucumber, and bean varieties. More studies are necessary to test the effect in commercial storage conditions.

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