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# ANTIFUNGAL ACTIVITY OF NANOPARTICLES AGAINST CHOSEN FUNGAL PATHOGENS OF CARAWAY

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**Abstract.** The paper shows the first study on the effect of nanoparticles against fungal pathogens of caraway. The fertilizers containing nanoparticles of copper, silver, and chitosan: Viflo® copper, Viflo Chitosol® Silver® Viflo Cal S and isolates KLM 1806 and KLM 1813 of *Septoria carvi* were used to the study. The percent of growth inhibition, development of *S. carvi* colonies and ability of Viflo® Cal to control plants against infection by *S. carvi* was adopted as the criterion of evaluation of the nanoformulations. The field experiment evaluated the effect of Viflo® Cal S to plant health in terms of the occurrence of *S. carvi* and *Erysiphe heraclei*, colonization of plant tissues by *S. carvi* and the yield of schizocarps. In vitro it has been shown that the tested formulations with nanoparticles limited the growth and development of the *S. carvi* and Viflo Cal® S at the concentration of 1 g$\cdot$cm$^{-3}$ was the inhibitor of mycelial growth. In climatic chamber Viflo Cal® S protect caraway's seedlings against the infection by *S. carvi*. *In vivo* Viflo Cal S® significantly limited the severity of septoriosis and powdery mildew, which contributed to increase the size and quality of the caraway crop.

**Key words:** caraway, fungi, control, nanopreparations

## INTRODUCTION

The occurrence of pathogenic fungi on caraway plants reduces the quantity and quality of herbal material [Machowicz-Stefaniak and Zalewska 2008]. Facultative parasites such as *Septoria* spp., *Colletotrichum* spp., *Phomopsis* spp. and *Phoma* spp. have great importance in causing caraway diseases in different geographical areas. Among the obligatory parasites species from the orders *Erysiphales* and *Pucciniales* are the

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most commonly reported ones [Grzybowska 1975, Mazur and Nawrocki 2004, Machowicz-Stefaniak and Zalewska 2008, 2011, Zalewska et al. 2015].

Considering the high harmfulness of these fungi, research on the factors limiting the growth and development of pathogens as well as plant protection on plantations is very important.

It was shown *in vitro* that fungicides Signum 33 WG, Curzate M 72.5 WP, Domark 100 EC, Sadoplon 75 WP, Dithane WG NeoTec 75 and Topsin M 500 SC completely inhibited the growth of *S. carvi*. On the other hand, preparations Biosept Active and Beta-Chikol limited the growth of the pathogen to 89.29% [Zalewska 2016]. *In vivo* studies confirmed the effectiveness of Biosept Active at the concentration of 0.1%, Beta-Chikol at the concentration of 2% and Dithane WG NeoTec 75 at 0.3% in reducing the occurrence of septoriosis on caraway plants in field conditions [Zalewska 2016].

In recent years interest has increased in the possibility of using preparations containing active substances in the form of nanoparticles to reduce the occurrence of bacteria and fungi pathogenic to humans and different crops [Feng et al. 2000, Jo et al. 2009, Kaur et al. 2012, Kim et al. 2012]. Preparations containing silver nanoparticles have been used for many years in disinfecting the equipment and medical rooms. In addition, it has been shown that they do not stimulate resistance in fungi, they are environmentally safe and inexpensive [Kim et al. 2012]. Studies of Kaura et al. [2012] demonstrated the possibility of using preparations containing chitosan and silver nanoparticles in reducing seed borne pathogens.

Considering the above information, the studies on the influence of preparations containing active substances in the form of nanoparticles on *Septoria carvi*, a dangerous pathogen of caraway were undertaken [Odstrčilová et al. 2002, Bedlan 2005, Machowicz-Stefaniak and Zalewska 2008, Mačkinaitė 2012].

## MATERIAL AND METHODS

Studies were carried out in 2013–2015. Three liquid foliar fertilizers containing nanoparticles of copper, silver and chitosan, produced by Vet-Agro Sp. z.o.o. in Lublin were included (tab. 1). These were the following formulations: Viflo® copper, Viflo® Chitosol Silver and Viflo Cal S – based on calcium, which in 2014 were awarded the A. Pieniążek prize as the most innovative product. Single spore cultures KLM 1806 and KLM 1813 of *Septoria carvi* obtained from the leaves of caraway with symptoms of septoriosis were selected from our own collection of isolates (phots 1, 2) [Machowicz-Stefaniak and Zalewska 2008]. Research conducted earlier *in vitro* and *in vivo* confirmed the high pathogenicity of these isolates towards caraway [Zalewska 2013].

**Evaluation of preparations *in vitro***. Experiments were performed on PDA (Difco) medium in two series, adding the studied formulations to the medium, using the method of poisoning the culture media and inoculated with agar plugs containing fungi [Machowicz-Stefaniak and Zalewska 2011]. The formulations were tested at three concentrations, i.e. concentration recommended in practice as well as lower and higher. Viflo® copper was tested at 230 $g \cdot cm^{-3}$ – recommended in practice as well as

100 g·cm$^{-3}$ and 10 g·cm$^{-3}$. Preparations Viflo Chitosol® Silver® and Viflo Cal S at the concentration of 0.125 g·cm$^{-3}$ – recommended in practice, and 0.05 g·cm$^{-3}$ and 1 g·cm$^{-3}$. The inoculum were the mycelia plugs of uniform size (3 mm) cut out from 2-week-old mother colonies growing on PDA medium at the temperature of 24°C. For each formulation at the tested concentration treated as an object and each fungus culture 4 replicates was included, treating the dish for repetition. The controls consisted of colonies of *S. carvi* growing on the PDA medium without preparations.

Table. 1. Characteristic of nanoparticles used in study

| Type | Physical form of preparation | Average particle size (μm) | Contents of nanoformulations (% or g·cm$^{-3}$) | Used solvent |
|---|---|---|---|---|
| Viflo® copper | blue colloid | 20–30 | 7.0% Cu* | pure water |
| Viflo® Chitosol Silver | light brown gel | 20–30 | 25 g·cm$^{-3}$Ag | pure water |
| Viflo® Cal S | light brown colloid | 20–30 | 25 g·cm$^{-3}$Ag; 6.0% CaO | pure water |

* – nanoparticles chelated with EDTA

Phot. 1. The first symptoms of caraway septoriosis

Phot. 2. Strong symptoms of septoriosis on caraway leaf

The measure of activity of the tested formulations was the percentage of growth inhibition of 4 and 8 day-old fungus colonies on the medium with preparation as compared to the control colonies [Machowicz-Stefaniak and Zalewska 2011].

$$I = \frac{C - T}{C} \times 100\%$$

where: I – inhibition rate, C – diameter of the control colony, T – diameter of the colony on the dishes with an addition of the preparations. Moreover, the type of toxic interaction of studied formulations on *S. carvi* was determined and in the absence of colony growth the inoculums were transferred to PDA medium without preparation to determine viability of the fungus [Machowicz-Stefaniak and Zalewska 2011]. Additionally, microscopic examination of 4 and 8 – day-old colonies of *S. carvi* growing on the medium with the preparations was also performed in order to determine changes in the appearance of morphological structures – hyphae, pycnidia and conidia.

**Evaluation of preparations *in vivo.* Studies in climate chamber.** Isolate KLM 1806 of *S. carvi* and formulation Viflo Cal® S at the concentration of 0.125 $g \cdot cm^{-3}$ –

recommended in practice and 1 g·cm$^{-3}$ – fungicidal in *in vitro* studies were chosen. For the fungus isolate, the tested concentrations and control, 50 of 6-week-old seedlings of caraway (total 150 seedlings) were prepared. The plants were obtained from schizocarps of caraway variety Konczewicki, which, after surface disinfection, were sown into pots 15 cm in diameter filled with sterile soil. The plants were kept in a climate chamber at the temperature of 24°C, 84% relative humidity and a 12-hour cycle of day and night, using Agro-Life fluorescent illumination.

Table 2. Comparison of average value of monthly temperature of air and rainfalls with average many years in vegetation periods in 2014–2015

| Month | Means of the years 1963–1992 | | Difference of mean air temperature in comparison with means of the years | | Percentage of the average annual rainfalls | |
|---|---|---|---|---|---|---|
| | air temperature (°C) | rainfalls (mm) | 2014 | 2015 | 2014 | 2015 |
| January | -3.8 | 26.2 | 2.4 | 4.55 | 188.93 | 167.17 |
| February | -2.9 | 27.3 | 4.6 | 3.5 | 75.46 | 101.83 |
| March | 1.1 | 27.1 | 1.9 | 3.5 | 214.02 | 124.72 |
| April | 7.4 | 40.4 | 2.3 | 0.2 | 88.12 | 43.06 |
| May | 13.3 | 54.4 | -0.1* | -0.9* | 223.52 | 88.73 |
| June | 16.5 | 68.9 | -1.3* | -0.4* | 104.50 | 89.55 |
| July | 18.0 | 78.3 | 2.15 | 1.5 | 207.92 | 65.26 |
| August | 17.1 | 73.7 | 0.4 | 4.6 | 129.03 | 16.15 |
| September | 12.9 | 47.3 | 1.8 | 2.2 | 67.65 | 154.12 |
| October | 7.8 | 41.0 | 1.95 | -0.3* | 93.42 | 79.76 |
| November | 2.5 | 40.9 | 2.95 | 2.2 | 73.84 | 147.92 |
| December | -1.4 | 33.8 | 2.1 | 7.9 | 109.76 | 29.3 |

* – temperature lower than mean of the years

Six-week-old seedlings of caraway were sprayed with the tested formulation at both concentrations, using a hand sprayer type Kwazar. Control plants were sprayed with sterile distilled water. Twenty-four hours after application of the product (Viflo Cal® S) the plants were sprayed with a spore suspension of *S. carvi* a density of $3 \times 10^6$/ml [Lamsal et al. 2011, Zalewska 2013]. Observations of plant health were carried out every 3 days for 3 weeks. After this time, 25 leaves were taken from each combination and mycological analysis was performed in order to reisolate the fungus used for the inoculation. For the tested concentrations of preparation and for control combination 100 leaf fragments of 3 mm were analyzed, as described by Machowicz-Stefaniak and Zalewska [2008].

**Field studies.** Under field conditions Viflo Cal® S formulation at the concentration of 0.125 g·cm$^{-3}$ was tested. As the criterion of preparation evaluation were used plant health, colonization of plants by *S. carvi* and *E. heraclei*, mycological analysis of above-ground parts of plant and size of the yield.

The study was conducted on a private 3.5 ha production plantation of caraway in the second year of cultivation located in the province Świętokrzyskie in the village Małoszyce: 50°51'15"N 21°27'03"E. In the earlier years of studies conducted on the plantations in the same village the symptoms of septoriosis on caraway plants were observed [Zalewska et al. 2015]. Four plots with an area of 0.1 are were separated on a the plantation. The preparation was applied twice, i.e. at the time of plant flowering and during schizocarps ripening. A preliminary estimation of the health was made before the first treatment, while the next ones before the second treatment and before schizocarps were harvested. For this purpose, the occurrence of septoriosis was estimated directly on the plantation according to Zalewska [2016].

A five-degree scale of infection was used, i.e. 0 – lack of symptoms, 1 – infection from 1 to 25% of the leaf area, 2 – infection from 26 to 50% of the leaf area, 3 – infection from 51 to 75% of the leaf area and 4 – infection over 75% of the leaf area, and then the disease indicator was calculated [Machowicz-Stefaniak 2010, Zalewska 2016]. During the evaluation of caraway health, the plant material, i.e. leaves, stems, umbels and schizocarps were taken for mycological analysis for the presence of *S. carvi* [Machowicz-Stefaniak and Zalewska 2008]. The presence of *E. heraclei* on caraway plants was included in study since the vegetation period of 2015 year favored the occurrence of powdery mildew [Zalewska et al. 2015]. To determine the rates of infection by *E. heraclei* a 4-degree scale was used: 0 – no symptoms, 1– infection to 25% of the leaf surface 2 – infection 25–75% of the leaf surface, 3 – infection over 75% of the leaf surface. Another criterion for estimating the preparation with nanoparticles of silver (Ag) was the caraway yield. For this purpose, the yield from the experimental plots was compared with the yield from the control plot as well as from the yield from 1 ha. Interpretation of the results was made with respect to the meteorological conditions surrounding Kielce published on-line [http://www.weatheronline.pl].

The obtained data of study were analyzed statistically using SAS® program.

## RESULTS

**Evaluation of preparation *in vitro.*** The study showed a varied effect of the tested nanoparticles of copper, silver and chitosan on the growth and sporulation of *S. carvi* colonies. Preparation Viflo® containing nanoparticles of copper significantly decreased the growth and development of 4-day-old colonies of the pathogen at the concentration of 230 $g \cdot cm^{-3}$, but at the concentration of 10 and 100 $g \cdot cm^{-3}$ the inhibition effect of the preparation was small. After 8 days of cultivation the inhibition effect of the preparation at the concentration of 100 $g \cdot cm^{-3}$ and 230 $g \cdot cm^{-3}$ of the growth of colonies was significantly higher in comparison with the growth of control colonies and the influence at the concentration of 10 $g \cdot cm^{-3}$, where the preparation stimulated the growth of colonies of *S. carvi* KLM 1806 isolate and inhibited the growth of colonies KLM 1813 isolate (tab. 3).

Similarly, preparation Viflo® Chitosol Silver limited the growth of 4 and 8-day-old colonies of *S. carvi* at a comparable level, i.e. to 50.9 and 48.57% respectively. However, the inhibitory effect of this preparation was significantly lower than the effect of Viflo® Cal S (tab. 3).

Table 3. Effect of foliar fertilizers containing nanoparticles on the growth inhibition of *S. carvi* colony on PDA

| | Izolaty | Percent of growth inhibition of 4-day-old colonies the content of nanoparticles (g·cm$^{-3}$) | | | | | Percent of growth inhibition of 8-day-old colonies the content of nanoparticles (g·cm$^{-3}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Viflo ® copper | | 10 | 100 | 230 | control | LSD | 10 | 100 | 230 | control | LSD |
| | KLM 1806 | 1.82 a | 9.09 a | 20.0 b | 0.0 a | 10.8003 | -6.67 a | 19.05 b | 25.71 b | 0.0 a | 7.2120 |
| | KLM 1813 | 0.0 a | 4.0 ab | 18.0 b | 0.0 a | 17.9833 | 11.88 b | 14.85 b | 31.68 c | 0.0 a | 10.3254 |
| | mean | 0.91 a | 6.55 ab | 19.0 b | 0.0 a | 14.3918 | 2.6 a | 16.95 b | 28.70 c | 0.0 a | 8.7687 |
| Viflo ® Chitosol Silver | | 0.05 | 0.125 | 1.0 | control | LSD | 0.05 | 0.125 | 1.0 | control | LSD |
| | KLM 1806 | 9.09 b | 5.45 ab | 50.9 c | 0.0 a | 8.5314 | 0.95 a | 4.76 a | 48.57 b | 0.0 a | 7.6604 |
| | KLM 1813 | 2.0 a | 2.0 a | 42.0 b | 0.0 a | 9.9980 | 0.0 a | 1.98 a | 39.60 b | 0.0 a | 10.8072 |
| | mean | 5.55 a | 3.73 a | 46.45 b | 0.0 a | 9.2647 | 0.48 a | 3.09 a | 44.09 b | 0.0 a | 9.2338 |
| Viflo® Cal S | | 0.05 | 0.125 | 1.0 | control | LSD | 0.05 | 0.125 | 1.0 | control | LSD |
| | KLM 1806 | 30.91 b | 43.64 c | 100.0 d | 0.0 a | 5.8348 | 23.81 b | 32.38 c | 100.0 d | 0.0 a | 2.0000 |
| | KLM 1813 | 30.0 b | 50.0 c | 100.0 d | 0.0 a | 9.0700 | 26.73 b | 47.52 c | 100.0 d | 0.0 a | 18.8700 |
| | mean | 30.45 b | 46.82 c | 100.0 d | 0.0 a | 7.4524 | 25.27 b | 39.95 c | 100.0 d | 0.0 a | 10.4350 |

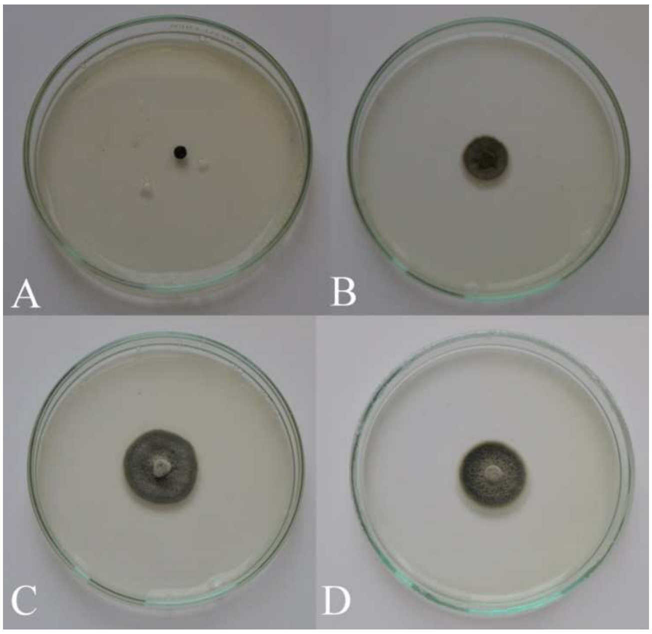
Values in lines marked with the same letter do not differ significantly, $p \leq 0.05$
LSD – low significant difference

Preparation Viflo® S Cal limited the growth and development of colonies of the studied isolates of fungus after 4 and 8 days of culture at all tested concentrations and at the highest concentration, i.e. 1 g·cm$^{-3}$ the growth of colony was inhibited in 100%. With the increase of the nanoparticles in the culture medium a significant rise of the percentage of colonies growth inhibition was observed (tab. 3).

Table. 4. The kind of toxic activity of nanoformulations on the growth of *S. carvi* colony

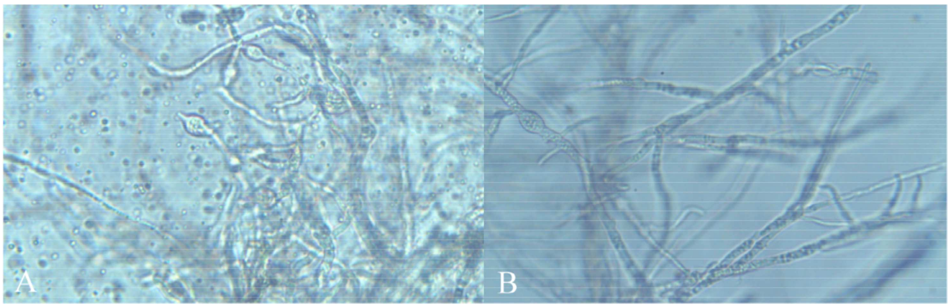
| | Isolates | Toxic activity | | |
|---|---|---|---|---|
| Viflo® copper (g·cm$^{-3}$) | | 10 | 100 | 230 |
| | KLM 1806 | ++ | + | + |
| | KLM 1813 | + | + | + |
| Viflo® Chitosol Silver (g·cm$^{-3}$) | | 0.05 | 0.125 | 1.0 |
| | KLM 1806 | + | + | + |
| | KLM 1813 | 0 | + | + |
| Viflo® Cal S (g·cm$^{-3}$) | | 0.05 | 0.125 | 1.0 |
| | KLM 1806 | + | + | – |
| | KLM 1813 | + | + | – |

– – fungicidal activity
+ – fungistatic activity
+ + – stimulating activity
0 – lack of toxic activity




Phot. 3. *Septoria carvi* KLM 1806 colonies on the PDA medium with silver nanoparticles – Viflo® Cal S in concentrations: 1 g·cm$^{-3}$ – A, 0.125 g·cm$^{-3}$ – B, 0.05 g·cm$^{-3}$ – C and control – D

The effect of preparation Viflo® with nanoparticles of copper on the colonies of *S. carvi* was fungistatic, except at the concentration of 10 g·cm$^{-3}$ while for Viflo® Chitosol Silver at 0.05 g·cm$^{-3}$ (tab. 4). Fungicidal properties towards *S. carvi* were shown only by Viflo® Cal S at the concentration of 1.0 g·cm$^{-3}$, but at the lower concentrations it was fungistatic (tab. 4, phot. 3).




Phot. 4. Lysis of *S. carvi* KLM 1806 mycelium caused by Viflo® Cal S at the concentration of 1 g·cm$^{-3}$ – A and control hyphae – B (×500)

Phot. 5. Deformation of *S. carvi* KLM 1806 hyphae on PDA medium with Viflo® Cal S at the concentration of 1 g·cm$^{-3}$ (×500)

The macroscopic observations of *S. carvi* colonies growing on the medium with nanoparticles showed that the colonies were compact, dark with a black reverse, like control colonies, but these colonies did not produce pycnidia after 4 and 8 days in contact with nanoparticles in contrast to control colonies. Microscopic studies showed that at the presence of Viflo® Cal S at the concentration of 1 g·cm$^{-3}$ the hyphae of *S. carvi* were degraded, lysed and dead (phot. 4.). On the other hand at the presence of Viflo® Chitosol Silver at thy concentration of 1 g·cm$^{-3}$, 0.1 g·cm$^{-3}$ and 0.05 g·cm$^{-3}$ and Viflo® Cal S at 0.1 g·cm$^{-3}$ and 0.05 g·cm$^{-3}$ the hyphae of the pathogen were deformed and did not form pycnidia (phot. 5).

**Evaluation of preparations *in vivo.* Studies in climatic chamber.** As a result of the application of a water suspension of *S. carvi* conidia on the leaves of 6-week-old seedlings of caraway, previously sprayed with preparation Viflo® Cal S, no symptoms of infection were observed until the 14$^{th}$ day of studies. In contrast, on control plants' leaves the first symptoms of septoriosis were observed 6 days after the inoculation and they constantly increased in size until the end of observation. They were small necrotic spots about the size of 2–3 mm, sunk in the tissue of leaves and petioles. Only two isolates of *Alternaria alternata* were obtained as a result of the mycological analysis of leaves treated with the test formulation and with the suspension of studied pathogen conidia. *Septoria carvi* was not reisolated from these leaves. However, in the control combination *S. carvi* constituted 96% of the isolated fungi. Macroscopic and microscopic features of *S. carvi* cultures corresponded to the features of the isolate used for inoculation.

**Field studies.** The starting observations of caraway health conditions conducted during the flowering period, i.e. on 23 May 2015 showed the presence of a single plant with symptoms of septoriosis on leaves. The indicators of leaf infection of plants on plots selected for control spraying and on control plots were low and were 0.35 and 0.39, respectively (tab. 5). Caraway plants were in good condition with good growth and coloration.

Table 5. The indicators of infection of caraway leaves by *Septoria carvi* during the growing season – spring/summer 2015

| Data of observation and spraying | | 23.05.2015 | 8.06.2015 | 23.06.2015 |
|---|---|---|---|---|
| Combination | Viflo® Cal S | 0.35 a* | 0.95 a | 1.5 a |
| | Control | 0.39 b | 9.81 b | 15.4 b |
| | LSD | 0.0582 | 0.8734 | 0.8625 |

* – differences in columns $p \leq 0.05$
LSD – low significant difference

The number of plants with symptoms of septoriosis did not increase during two weeks after the first treatment of control, but the increase in the number of leaves with disease symptoms was observed and it resulted in an increase of infection indicators to 0.95 on the sprayed plots and to 9.81 in the control combination (tab. 5). After the second protective treatment the index of leaf infection by *S. carvi* increased slightly and amounted to 1.5. However, in the control combination there was a significant increase of septoriosis because the indicator of infection was 15.4 (tab. 5).

During the observation of the healthiness of caraway biennial plants in summer months, i.e. May, June and July of 2015, there was a very low percentage of normal rainfall not exceeding 89%, and the mean monthly temperature was lower than average long-term air temperature (tab. 2). However, in June there were periods with a few day of very high temperature above 25$^{o}$C.

Table 6. Fungi isolated from various organs of caraway (*Carum carvi* L.) plants treated with Viflo® Cal S and from control combination in 2015

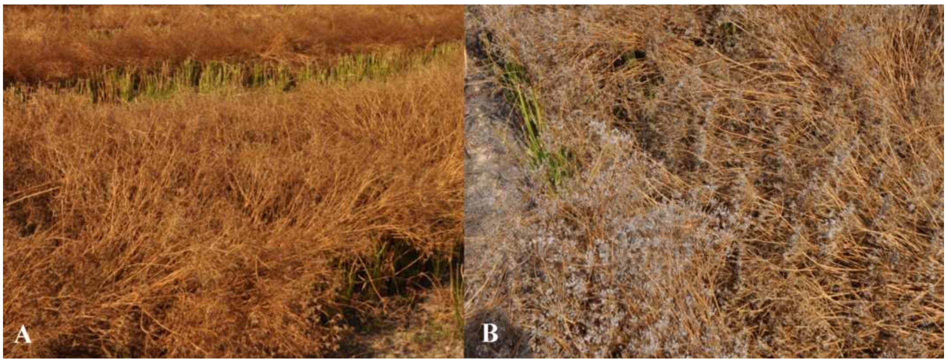
| Fungi | Number of isolates term of isolation | | | |
|---|---|---|---|---|
| | I | | II | |
| | before use of Viflo® Cal S | control | after two spraying with Viflo® Cal S | control |
| *Septoria carvi* Syd. | 32 | 35 | – | 21 |
| Other fungi | | | | |
| *Alternaria alternata* (Fr.) Keissl. | 48 | 36 | 40 | 42 |
| *Alternaria radicina* Meier Drechsler et E.D. Eddy | 14 | 22 | 10 | 32 |
| *Botrytis cinerea* Pers. | 22 | 19 | – | 16 |
| *Cladosporium cladosporioides* (Fresen.) G.A. de Vries | 27 | 21 | – | 34 |
| *Colletotrichum gloeosporioides* (Penz.) Penz. et Sacc. | 7 | – | 5 | 3 |
| *Epicoccum nigrum* Link | – | 12 | 2 | 4 |
| *Fusarium equiseti* (Corda) Sacc. | – | – | – | 4 |
| *Fusarium oxysporum* Schlecht. | – | 2 | – | – |
| *Fusarium poae* (Peck) Wollenw. | 2 | 11 | – | 10 |
| *Fusarium sporotrichioides* Sherb. | 2 | – | 4 | 2 |
| *Giberella avenacea* R.J. Cook | – | – | 4 | 2 |
| *Sclerotinia sclerotiorum* (Lib.) de Bary | – | 2 | – | 2 |
| Total other fungi | 154 | 160 | 65 | 172 |

I – first term of isolation, i.e before use preparation
II – second term of isolation , i.e. after double use of preparation

Mycological analysis of above-ground parts of caraway carried out before protection treatment showed that they were colonized by various species of fungi (tab. 6). *Septoria carvi* was isolated from all above-ground organs of caraway and especially from the leaves and petioles. After the second application of nanoparticles the fungus *S. carvi* was not isolated, but cultures of other phyllosphere species of fungi were isolated (tab. 6). However, their number and differentiation of species were significantly smaller than the number and variety of fungi obtained from control plants. Similarly, a lot of *S. carvi* isolates were obtained from control plants in that time.

The study on the effect of foliar fertilizer Viflo® Cal S, which contains silver nano-particles, on the quality and quantity of yield of caraway schizocarps showed its rise from 0.15 to 0.30 kg/m$^2$, i.e. from 1 to 2%. Moreover, schizocarps obtained from control plants with nanopreparation were well formed, large and pycnidia of *S. carvi* and chasmothecia of *Erysiphe heraclei* did not occur on their surface in contrast to schizocarps of not treated plants.


Phot. 6. Caraway plants sprayed with Viflo® Cal S – A and control plants – B with symptoms of *Erysiphe heraclei* during the growth of plants




Phot. 7. Caraway plants sprayed with Viflo® Cal S – A and control plants – B with symptoms of *Erysiphe heraclei* during harvest

The first symptoms of powdery mildew caused by *E. heraclei* were observed in the middle of June. A soft, white and powdery coat was observed on the stems, leaves, umbels and schiozocarps of 60% plants and on the plantation (phot. 6). The above-mentioned symptoms were not observed on plants sprayed with the studied nonopreparation Viflo® Cal S. During the third observation of plant healthiness conducted just before harvesting of schizocarps, the symptoms of powdery mildew occurred on all plants in the control combination. However, in a combination with nanopreparation Viflo® Cal S those symptoms were observed with much less intensity on about 15% of plants (phot. 7). Moreover, distinct degradation of the pathogen mycelium and conidia was observed in microscopic preparations.

## DISCUSSION

Many years of research on the diseases of caraway indicate that the pathogenic fungi can occur epidemically, which leads to large economic losses and even unprofitability of this crop. A high threat for caraway crops caused by *S. carvi* was shown recently. The fungus appears periodically on plants in the first and second years of cultivation during the conditions of increased relative humidity and high temperature [Machowicz-Stefaniak and Zalewska 2008, Zalewska et al. 2015]. Similarly, during the period with high temperature *E. heraclei* can occur epidemically [Zalewska et al. 2015]. Therefore, adequate plant protection of caraway is very important to limit the infection and occurrence of pathogenic fungi.

Great effectiveness of preparations Biosept Active and Beta-Chikol in causing growth increasing resistance of plants to infection, in limiting the growth and development of *S. carvi* and other pathogens *in vitro* was shown recently [Zalewska 2016]. However, the effect of these preparations *in vivo* was not sufficient because in spite of their application the infection occurred and pycnidia as well as conidia of *S. carvi* were formed on septoriosis spots on the leaves [Zalewska 2016]. According to Thomson and Water [2007], the protection of caraway using fungicides based on azoxystrobin (Quadris) is adequate when the first control application is performed just at the beginning of florescence. Starting the protection of plants at the stage when 50% of the inflorescence bloom does not increase the yield. However, the study carried out by Odstrčilová [2007] on the use of fungicides in caraway control after flowering caused reduction of pathogenic fungi, i.e. *Ascochyta carvi* and *Septoria carvi*, but did not influence limitation of contamination of seeds by saprophytic fungi.

Due to their purpose, spice and medicinal plants should be free of pathogenic fungi and their toxic secondary metabolites, as well as free from chemical residues [Jampílek and Kráľová 2015]. However, preparations used to control them should be safe for humans. Such preparations include those containing active substances in the form of nanoformulations. Silver ions (Ag+) have been used for a very long time in medicine as disinfectants against a variety of microorganisms, and as formulations to facilitate wound healing [Kim et al. 2012]. Studies of Jung et al. [2008] demonstrated a destructive effect of silver ions in the form of nanoparticles towards bacteria *Staphylococcus aureus* and *Escherichia coli*, which are very harmful to humans. The silver ions caused growth inhibition of bacteria, damage of cytoplasmic membranes, separation of the cell membrane from the cell wall and destruction of the cell wall resulting in lysis of the bacterial cells. The use of such formulations in plant protection is desirable also because of their favourable interaction with other non-target organisms that directly affect the fertility of the soil [Jampílek and Kráľová 2015]. The use of such nanoformulations is friendly to the environment, and when used in appropriately selected concentrations they do not accumulate in organisms and the environment [Jampílek and Kráľová 2015].

In literature there is no information on the effect of nanoformulations for fungi pathogenic to caraway. *In vitro* studies showed a significant inhibition of the growth of *S. carvi* colonies growing on the medium containing active substances in the form of nanoparticles. It was shown that the inhibition of the pathogen growth increased with the growth of the concentration of active substances in the culture medium, which was observed for other fungi by [Ogar et al. 2015]. The limitation of the fungus colony growth was probably the result of degradation and lysis of hyphae, especially after using Viflo® Cal S tested at the concentration of 1 $g \cdot cm^{-3}$. The inhibitory properties of formulation Viflo® Cal S were also observed *in vivo*, i.e. in climatic chamber and in the field plantation of caraway both towards facultative and obligatory parasites. That was indicated by the lack of symptoms of septoriosis on plants sprayed with Viflo Cal S in the climatic chamber and in the field plantation, as well as by the lack of colonization of plants by *S. carvi* and much less microbial contamination of plants by associated phyllophere fungi contrary to the control combination. Similarly, the inhibition properties of preparations containing silver nanoparticles were shown in a study conducted by Kim et al. [2012] for several species of pathogenic fungi of *Alternaria*, *Botrytis*, *Cladosporium*, *Cylindrocarpon*, *Fusarium*, *Glomerella* genera and others. The inhibition effect increased with the increase of the concentration of the nanoparticles in the medium [Kim et al. 2012]. The destructive effect of silver nanoparticles was also shown in studies of Min at al. [2009] towards the sclerotia forming fungi, i.e. *Sclerotinia sclerotiorum*, *S. minor* and *Rhizoctonia solani*. The microscopic studies of these species mycelium treated with nanoformulations showed severe damage involving the separation of the membrane from cytoplasm, causing lysis of the cells and no possibility of germination of sclerotia [Min at al. 2009]. Moreover, the present study demonstrated the inhibiting effect of Viflo® Cal S to *E. heraclei*, which corresponding to the study by Lamsal et al. [2011] who showed strong inhibiting effect *in vivo* of silver ions to the mycelium and spores of the pathogen causing powdery mildew of cucurbits. The mechanism of the inhibitory action of silver ions towards fungal cells is not yet fully understood. However, it is known that silver inhibits the metabolism of fungal and bacterial cells, it affects the function of membrane-bound enzymes, especially those in the respiratory chain and cause physical damage. Silver ions accumulate in vacuoles and cause their rupture and spillage of the contents. Besides, they generate reactive oxygen species (ROS) by reaction with oxygen, which leads to the damage of cell wall proteins, fats and nucleic acid [Thurman et al. 1989, Hwang et al. 2008, Jampílek and Kráľová 2015]. In recent studies of Ogar et al. [2015] the inhibition effect of silver nanoparticles was shown towards the moulds commonly occurring in urban environments that cause allergies and various toxic symptoms of people. Additionally, in conditions of high humidity they may grow on the walls of buildings. These studies showed not only limitation of the growth of the fungi colonies on the medium containing silver ions proportionally to the increase of their concentration but also changes in the size of morphological structures of these fungi, i.e. conidiophores and spores and changes of colony colour resulting from the reduction of melanin production. Interestingly, studies of Ogar et al. [2015] showed stimulation of the development of certain species of fungi by silver ions, which was shown for *Mortierella alpine* a very dangerous species, producing toxins and affecting a lot of plant species.

In the present study the stimulating effect towards one of *S. carvi* isolate was shown for preparation Viflo® containing nanoparticles of copper at the concentration of 10 g·cm$^{-3}$. A stimulating interaction of copper in the form of fungicide was shown in previous studies in relations to *S. carvi* [Zalewska 2016] and *Monilia coryli*, the fungus causing brown root of hazelnut [Zalewska 2005].

In the present study the inhibition effect was also shown for the preparation Viflo® Chitosol Silver containing silver and chitosan nanoparticles. However, the efficacy of this nanopreparation was much lower than the efficacy of nanoparticles containing only silver nanoparticles. Furthermore, in microscopic preparations the deformation of hyphae was observed occasionally, but plasmolysis of hyphae was not observed. In contrast, the studies of Kaur et al. [2012] indicated a more efficient inhibitory effect of silver nanoparticles in combination with nanoparticles of chitosan towards *Aspergillus flavus*, *Alernaria alternata* and *Rhizoctonia solani* colonies than of these two factors separately. Moreover, the effect of silver and chitosan nanoparticles used in the same single formulation was comparable to the preparation used in medicine showing antifungal properties, i.e amphotericin – B.

The conducted research and its results indicated the desirability of testing nanoformulation Viflo® Cal S in field condition on caraway plantations in subsequent years. Such studies conducted *in vivo* will help to determine the most effective conditions for nanoparticles in reducing pathogenic fungi in caraway control.

## CONCLUSIONS

1. All tested nanoparticles *in vivo* limited in various degrees the growth and development of *Septoria carvi;* however, only Viflo® Cal S at the concentration of 1 g·cm$^{-3}$ totally inhibited the mycelial growth.

2. In conditions of the climate chamber Viflo® Cal S at the concentration of 0.125 g·cm$^{-3}$ and 1 g·cm$^{-3}$ protected seedlings of caraway against infection by *S. carvi.*

3. *In vivo* Viflo® Cal S significantly limited the severity of septoriosis and powdery mildew in comparison to the severity of these diseases in the control plants, which contributed to an increase in the size and quality of the crop of caraway schizocarps.

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## ODDZIAŁYWANIE NANOPREPARATÓW NA WYBRANE GATUNKI GRZYBÓW PATOGENICZNYCH DLA KMINKU ZWYCZAJNEGO *Carum carvi* L.

**Streszczenie.** W pracy przedstawiono pierwsze badania nad oddziaływaniem nanopreparatów na grzyby patogeniczne dla kminku zwyczajnego. Uwzględniono nawozy dolistne zawierające nanocząsteczki miedzi, srebra i chitozan: Viflo® miedziowy, Viflo® Chitosol Silver, Viflo® Cal S oraz izolaty KLM 1806 i KLM 1813 *Septoria carvi*. Za kryterium oceny preparatów przyjęto procent zahamowania wzrostu i rozwoju kolonii *S. carvi*, zdolność Viflo® Cal S do ochrony roślin przed infekcją *S. carvi.* W doświadczeniu polowym oceniano wpływ Viflo® Cal S na zdrowotność roślin w aspekcie występowania *S. carvi* i *E. heraclei*, zasiedlania tkanek roślinnych przez *S. carvi* oraz na wielkość plonu. *In vitro* wykazano, że testowane nanopreparaty ograniczały wzrost i rozwój *S. carvi*, a Viflo® Cal S w stężeniu 1 $g \cdot cm^{-3}$ był inhibitorem rozwoju grzybni. W warunkach fitotronowych chronił siewki kminku zwyczajnego przed infekcją *S. carvi*. W warunkach naturalnych Viflo® Cal S ograniczał istotnie nasilenie septoriozy oraz mączniaka właściwego, co przyczyniło się do zwiększenia wielkości i jakości plonu rozłupek kminku zwyczajnego.

**Słowa kluczowe:** kminek zwyczajny, grzyby, zwalczanie, nanopreparaty

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