https://doi.org/10.17221/492/2023-PSE

Bioproducts and their potential in protection of *Brassica napus* L. against *Verticillium longisporum*

Eva Zusková, Veronika Konradyová, Pavel Ryšánek, Jan Kazda*

Department of Plant Protection, Faculty of Agrobiology Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

**Corresponding author: zuskovae@gmail.com*

Citation: Zusková E., Konradyová V., Ryšánek P., Kazda J. (2024): Bioproducts and their potential in the protection of *Brassica napus* L. against *Verticillium longisporum*. Plant Soil Environ., 70: 188–194.

Abstract: The experiments were carried out on 5 different bioproducts for control of *Verticillium longisporum* in oilseed rape. For *in vitro* trials, greenhouse trials and field trials, the bioproducts used were based on bacteria (*Bacillus amyloliquefaciens*, *Pseudomonas veronii*) and fungi (*Pythium oligandrum*, *Trichoderma asperellum*, *Coniothyrium minitans*). In *in vitro* trials, some products (based on *Trichoderma asperellum*) showed a 100% inhibition effect on the pathogen for a whole trial period of 45 days. The greenhouse trial showed significant differences in bioproducts effectiveness ($P < 0.00723$) compared to control. Three bioproducts (based on *Coniothyrium minitans*, *Trichoderma asperellum* and *Pseudomonas veronii*) showed a 100% inhibition effect against the pathogen. In field trials conducted in 3 locations, there were some differences in yield, which can be important for growers and practice use. The highest yield (19.1% higher than the control) was achieved with a bioproduct based on *Trichoderma asperellum*. In trials, it is possible to see that there are promising results that can be used for further testing.

Keywords: fungal pathogen; premature ripening; spores; biological control agents

Verticillium longisporum differs from other species by not causing wilt symptoms but inducing premature ripening (Hornig 1987, Knüfer 2011) and *Verticillium* stem striping (Depotter et al. 2016). In the field, the first symptoms of the disease occur relatively late, at the beginning of plant ripening (Zeise and Seidel 1990). Early symptoms can be discolourations of stems, such as brownish stripes on one side along the stem, and later microsclerotia becomes visible beneath the epidermis, in the pith and the root tissue (Knüfer 2011). Oilseed rape responds to pathogen invasion with the formation of vascular occlusions, which affect water transport in the plant (Eynck 2008).

Microsclerotia starts to germinate in the soil when attracted by root exudates of the plant (Schnathorst 1981). Studies have demonstrated that microsclerotia can be stimulated by the host and nonhost root exudates (Schnathorst 1981, Mol and Riessen 1995). Exudates released by root cells diffuse into the rhizosphere and initiate an exudate gradient (Olsson and Nordbring-Hertz 1985), which induces the movement of hyphae toward the root. The parasitic phase of the fungus is initialised by direct penetration of the root epidermal cells (Zhou et al. 2006, Eynck et al. 2007). *V. longisporum* spreads in the vascular system and exhibits an extended latency phase in the stems, during which infection remains symptomless (Eynck et al. 2007). When there are not enough nutrients in the xylem, the pathogen leaves the xylem vessels and enters the parenchymatic cells (Knüfer 2011). After root infection, xylem invasion, and colonisation of stems, *V. longisporum* forms microsclerotia, which remains viable for several years in soil (Zheng et al. 2019) and causes long-lasting soil contamination (Heale and Karapapa 1999).

Protection is quite difficult due to the long persistence of the pathogen in the soil, as well as the

Supported by the Ministry of Agriculture of the Czech Republic, Project No. 270-2021-18111.

https://doi.org/10.17221/492/2023-PSE

wide range of hosts, mostly plants in the Brassiceae family (Depotter et al. 2016). Once the pathogen has spread in the vascular bundles of the plant, any protection is ineffective, so the aim is to reduce the content of the primary inoculum in the soil as much as possible. Zheng et al. (2020) surveyed diseases and pests in oilseed rape, and *V. longisporum* was evaluated as one of the 16 most severe diseases in oilseed rape worldwide.

The interest in biopesticides in Europe is increasing, partly due to increased demand for environmentally safe plant protection products. In the Czech Republic, as in all European Union countries, there are strategies for farming to fork and biodiversity, which bind the EU to achieve a 25% share of organic farming by 2030. According to Market and Markets™ (market research platform, presented in 2022), the biopesticide market is expected to grow to 13.9 billion USD by 2028. Other potential control methods of *Verticillium longisporum* in oilseed rape are except microorganism also cross-protection with non-pathogenic lineage A1/D2 of *V. longisporum* (Vega-Marin and Tiedemann 2022).

Therefore, we have targeted our research on biopesticides. We hypothesised in this experiment that there are bioproducts with the active substance of bacteria or fungi, which can be antagonistic against the pathogen *Verticillium longisporum*, per genus *Verticillium*.

MATERIAL AND METHODS

Biological material. Several bioproducts commonly used among farmers have been used for experiments (Table 1).

Verticillium longisporum VL43, isolated from *Brassica napus* in Northern Germany (University of Göttingen collection), was used for *in vitro* and greenhouse trials. Strain VL43 was selected based on pilot testing, where it showed the highest virulence (other tested strains from the CBS database 110222, 110232, 110277, 124.64, 128316, 128317).

For the greenhouse trial, the oilseed rape cv. Inspiration was chosen based on the long-term observation by the Union of Oilseed Growers and Processors (UOGP) as one of the most vulnerable cultivars to *V. longisporum*. Moreover, cv. Inspiration was one of the most grown cultivars at the time.

Oilseed rape cv. Alicante was chosen for field trials because cv. Inspiration was not available on the market. Alicante was a widespread cultivar among farmers without any potential advantage against the pathogen (based on the long-term observation of UOGP).

***In vitro* trials.** A strain of *Verticillium longisporum* VL43 grown on Potato Dextrose Agar (PDA) was used for a preliminary antifungal activity test. Five bioproducts, each as one variant (with 10 repetitions) of the experiment and control, were used in this test. *Verticillium longisporum* was inoculated on the right side of the Petri dish and left to grow for one week before bioproducts were applied (due to a slow growth of the pathogen). The bioproduct amount used for testing was calculated based on the Petri dish's size and the etiquette recommendation (the highest amount of preparation and water to fit manufacturer recommendations was used). The Petri dishes were stored at 21 °C in the dark for the duration of the experiment. The growth of mycelium was measured 45 days after bioproduct application (every 15 days), and the percentage inhibition was calculated.

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

where: C – colony diameter (mm) of the control; T – colony diameter (mm) of the test plate (Bekker et al. 2006).

Greenhouse trials. In greenhouse trials, it was necessary to consider other conditions that are more similar to practice. The experiment was kept at a stable temperature of 21 °C in pots 9 × 9 cm. Cv. Inspiration with 5 bioproducts was used. For each bioproduct and control, there were 3 repetitions with 10 plants.

At the beginning of the experiment, soil substrate (profi substrate Gramoflor) and seeds were sterilised

Table 1. Bioproducts and their active substances used *in vitro*, greenhouse and field trials

Bioproduct	Active substance	Producer
Hirundo	*Bacillus amyloliquefaciens* FV08-10	Monas technology
Prometheus	*Pseudomonas veronii* CCM 9674	
Polyversum	*Pythium oligandrum*	Biopreparáty
Xilon	*Trichoderma asperellum* T34	Kwizda Agro
Contans	*Coniothyrium minitans*	AgroProtec

https://doi.org/10.17221/492/2023-PSE

(2 min in 70% ethanol), and spores 8 × 10^3 in 1 mL suspension was prepared. Five days after sowing, 1 mL of the microsclerotia suspension was pipetted in the middle of the pot (1 cm deep). The day after, a solution of biological preparation was pipetted (in the same place). As recommended on etiquette, the solution was calculated for a pot 9 × 9 cm in size (the highest amount of preparation and water to fit manufacturers' recommendations was used). The experiment was carried out over 6 weeks (from bioproduct application).

Experiment evaluation. Ten samples (the whole plant at BBCH 14–16) were collected from each trial variant to evaluate the amount of *Verticillium longisporum*. All plants were freed of surface impurities, then immersed in ethanol (70%) for 2 min, then in 30% bleach solution (1.5% NaOCl, sodium hypochlorite 4.7 g/100 g = 100%) for 10 min, washed twice with distilled water. The bottom half of the plant (including the root neck) was used for real-time PCR (polymerase chain reaction) diagnosis.

Field trials. The experiment occurred at three localities, Chlumec nad Cidlinou, Trutnov and Kujavy, in the 2019/2020 season. At each locality, there were 3 repetitions in each variant (each repetition had approximately 15 m^2), and 9 samples per bioproduct were available for evaluation. The methodology was set up according to the requirements of used biopesticides (Table 2). Bioproducts with *Pythium oligandrum* (Polyversum) were also tested for possibilities of different applications. Bioproducts based on *Pseudomonas veronii* CCM 9674 (Prometheus) and *Bacillus amyloliquefaciens* FV08-10 (Hirundo) were used concerning specific conditions that are necessary for effectiveness (pH and amount of Ca in soil). Other treatments of the stand followed good agronomic practice.

Localities description. Localities were chosen in cooperation with UOGP based on their geographical differences and discussion with experimental stations about the possible presence of *Verticillium longisporum*. Rainfall and temperatures at the localities were typical for the geographic location.

Trutnov (50°33'41.4"N, 15°52'55.0"E). The locality is 450 m a.s.l., sandy loam, average temperature 7.2 °C, annual rainfall 708 mm. Preceding crops were fodder peas (2019) and winter wheat (2018). The value of pH at the locality is 5.9. Amount of nutrients at locality: P 153 mg/kg, K 174 mg/kg, Mg 174 mg/kg, Ca 1 353 mg/kg. Land preparation before sowing (29. 8. 2019) tillage, preparation with a cultivator.

Chlumec nad Cidlinou (50°07'46.3"N, 15°30'41.7"E). The locality is 240 m a.s.l., clayey, average temperature 8.7 °C, annual rainfall 642 mm. The preceding crops were winter wheat (2019) and fodder bean (2018). The value of pH at the locality is 6.8. Amount of nutrients at locality: P 63 mg/kg, K 300 mg/kg, Mg 145 mg/kg, Ca 4 413 mg/kg. Land preparation before sowing (31. 8. 2019) stubble – ploughing, deep undermining, 2 × preparation with cultivator Verso.

Kujavy (49°41'24.9"N, 17°58'24.8"E). The locality is 260 m a.s.l., sandy, average temperature 8.25 °C,

Table 2. Overview of field trials methodology at 3 localities. Specific of bioproduct application rates and plant phenological phase of treatment

Variant	*Coniothyrium minitans* – Contans	*Pythium oligandrum* – Polyversum				*Trichoderma asperellum* – Xilon	*Pseudomonas veronii* – Prometheus	*Bacillus amyloliquefaciens* – Hirundo	Control – Alicante
		1	2	3	4				
35 week 2019									
Before sowing	2 kg/ha	×	×	×	×	×	×	×	×
Pickling	×	5 g/kg	5 g/kg	×	×	×	×	×	×
During sowing	×	×	×	×	100 g/ha	10 kg/ha	×	×	×
42 week 2019									
BBCH 14–16	×	×	100 g/ha	100 g/ha	×	×	1 L/ha	1 L/ha	×
11–14 week 2020									
BBCH 32	×	×	×	×	×	×	1 L/ha	1 L/ha	×
17–18 week 2020									
BBCH 65	×	×	×	×	×	×	1 L/ha	1 L/ha	×

https://doi.org/10.17221/492/2023-PSE

annual rainfall 604 mm. Preceding crops were spring barley (2019) and sugar beet (2018). The value of pH at the locality is 6.5. Amount of nutrients at locality: P 100 mg/kg, K 205 mg/kg, Mg 140 mg/kg, Ca 2 095 mg/kg. Land preparation before sowing (29. 8. 2019) stubble-tillage, tillage, preparation with cultivator.

Experiment evaluation. Five plants were randomly chosen (diagonal every other 3–4 plants) from each plot in each repetition after harvest (15 plants for each variant). For real-time PCR analysis, a stem about 10–20 cm from the root neck was used (Knüfer 2011). Yield data was collected for each locality.

Detection and determination of *Verticillium longisporum*. Real-time PCR was used to evaluate greenhouse and field experiments. Plants or plant parts were frozen to –80 °C and lyophilised. Each sample was analysed using the greenhouse experiment. A mixed sample was assembled from five plants (one plot) in the field trials. The plant material was crushed in a mill (Retsch®, MM400, 50 mL steel grinding jars, 1 × 25 mm steel ball, 2 min at 30 Hz). A 0.1 g sample was weighed from the crushed plant material, from which total DNA was isolated by the phenol-chloroform isolation method (Barker 1998). The DNA concentration in the sample was measured (ThermoScientific, NanoDrop 2000), and the samples were used to analyse the amount of *Verticillium longisporum* DNA by real-time PCR. Oligonucleotide primers OLG70/OLG71 were used for real-time PCR, targeting the ribosomal internal transcribed spacer (ITS) region (Eynck et al. 2007).

OLG 70 forward CAGCGAAACGCGATATGTAG
OLG 71 reverse GGCTTGTA GGGGTTTAGA

The predicted length of PCR products is 261 bp.

Real-time PCR reactions were performed using the Bio-Rad CFX 96 real-time PCR detection system to assess fungal DNA quantification. The 10 µL reaction mixture consisted of 1 µL total DNA and 0.2 µL of primer mix (concentration of primers was 1:1:2 – R:F:*dd* water), Master mix 5 µL (Applied Biosystems, Syber Select Master Mix). Each DNA sample was quantified in three (primer sensitivity) or two (fungal DNA quantification) technical replicates from which average starting quantity values were calculated. The PCR program consisted of a 3 min activation step at 95 °C, followed by 40 cycles with 30 s at 95 °C, 30 s at 58 °C and 30 s at 72 °C, and a final elongation step of 2 min at 72 °C. The quantity of fungal DNA in plant tissue was determined based on a standard curve developed from a 10-fold dilution series (1 000 to 0.0001 pg) of purified *V. longisporum* isolate VL43 DNA (Eynck et al. 2007 modified). Modifications in the PCR program were based on pretests of different temperature gradients, and the best option was chosen for diagnosis.

Statistical processing. The normality of all sets of data was tested first. For *in vitro* evaluation, 2D graph line plot variables were used. In the greenhouse experiment, differences between each variant were tested using one-way ANOVA with a post-hoc Bonferroni test. In field trials, differences in yield in each locality were tested separately by one-way ANOVA. All statistic data was processed in Statistica 14 by TIBCO Statistica™ (Santa Clara, USA) in a 95% confidence interval.

RESULTS AND DISCUSSION

***In vitro* testing.** The bioproduct based on *Trichoderma asperellum* T34 (Xilon) shows the best results *in vitro*, with no pathogen mycelial growth. It clearly shows 100% effectivity in preventing pathogen growth (Figure 1), as *Trichoderma asperellum* grew over the whole Petri dishes (Figure 2).

Trichoderma asperellum T34 shows a 100% inhibition effect during the whole experiment. Furthermore, *Pseudomonas veronii* CCM 9674 (Prometheus) looks promising, with inhibition over 80%. *Pythium oligandrum* (Polyversum) had a slower response to the pathogen in the first few days but eventually achieved 94.56% inhibition. In the rest of the bioproducts, antifungal activity peaked after 30 days and then decreased.

Greenhouse experiments. In the greenhouse experiment with the cv. Inspiration, there are significant differences (P = 0.00723) between the control and all other bioproducts. All the bioproducts showed a certain level of effectiveness against *V. longisporum*.

Trichoderma asperellum T34 (Xilon), *Pseudomonas veronii* CCM 9674 (Prometheus), and *Coniothyrium minitans* (Contans) showed no amount of *V. longisporum* in the samples. There was measured *V. longisporum* in all samples with *Bacillus amyloliquefaciens* FV08-10 (Hirundo) (mean = 0.9681; standard deviation = 0.0068) and control (mean = 264.1284; standard deviation = 418.4166) and in 6 samples with *Pythium oligandrum* (Polyversum) (mean = 0.5451; standard deviation = 0.4607).

Field trials. There are differences in the amount of pathogen between variants and localities with magnitudes of $1–10^3$, even though no statistically significant differences (P = 0.205) exist between treatments (Table 3).

https://doi.org/10.17221/492/2023-PSE

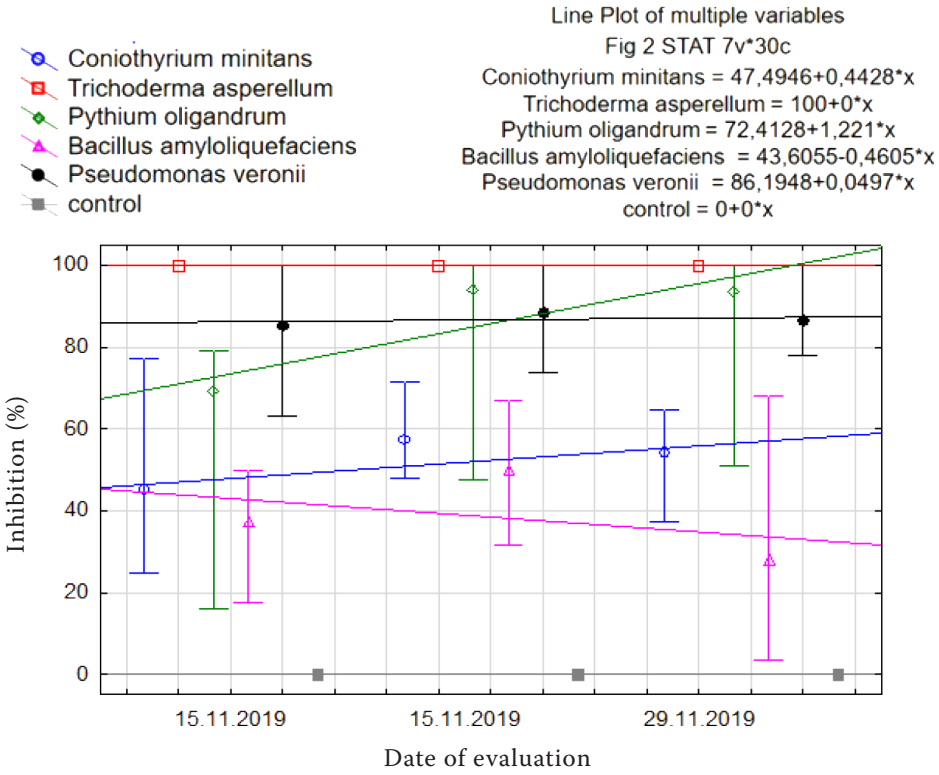

Figure 1. *In vitro* testing – inhibition of mycelia growth for 5 bioproducts over 45-day period. Trends of the growth with average and standard deviation

At the locality Kujavy, there was just one plot from which samples with the pathogen *Verticillium longisporum* were collected. At the other localities, the occurrence of pathogens was more common. As pathogen inoculum was not used for experiments (natural conditions), findings were irregular. Zheng et al. (2019) reported that *Verticillium longisporum* was found in previously uninfected areas and even in regions with a short history of oilseed rape cultivation. Zheng et al. (2019) assumed that the reason could be seed transmission (not confirmed), as *Verticillium* stem striping is present in disease hot spots. Based on our results, we assume that hot spots can be found even in single areas in the locality. Hot spots of high levels of the pathogen in soil at one locality have already been reported by Tenuta (2019).

Yield shows no differences between treatments (P = 0.861) without regard for locality. Even with no statistical differences, percentage differences in yield (Figure 3) are useful in practice and important for farmers. At locality Chlumec nad Cidlinou, the highest yield was achieved by the variant treated by *Trichoderma asperellum* T34 (Xilon); the difference between the lowest and highest yield was 19.1%. At locality Trutnov, the highest yield was obtained from the variant treated by *Pseudomonas veronii* CCM 9674 (Prometheus), which was 8.7% higher than the control (lowest yield). Lastly, at locality Kujavy, the highest yield was achieved by the variant treated by *Pythium oligandrum* 4 (Polyversum – bioproduct application during sowing), 9.6% higher than the control (lowest yield). Differences such as 19% are promising for use in practice.

Various bioproducts were tested as possible treatments against *V. longisporum* in field conditions. We showed in experiments that there is some level of effectiveness in all bioproducts against *V. longisporum*. Bioproducts based on *Trichoderma asperellum* T34 especially showed good results in the experiments. Even with no statistical differences in the field trials, it is still possible to assume that there are some differences in treatments (Figure 3). However, factors other than treatment may influence the amount of pathogens in plants (rainfall, temperature, type of soil, bioactivity in the soil, etc.). Furthermore, there is a need to conduct research into different methods, times, and frequency of bioproduct application concerning soil and weather conditions.

https://doi.org/10.17221/492/2023-PSE

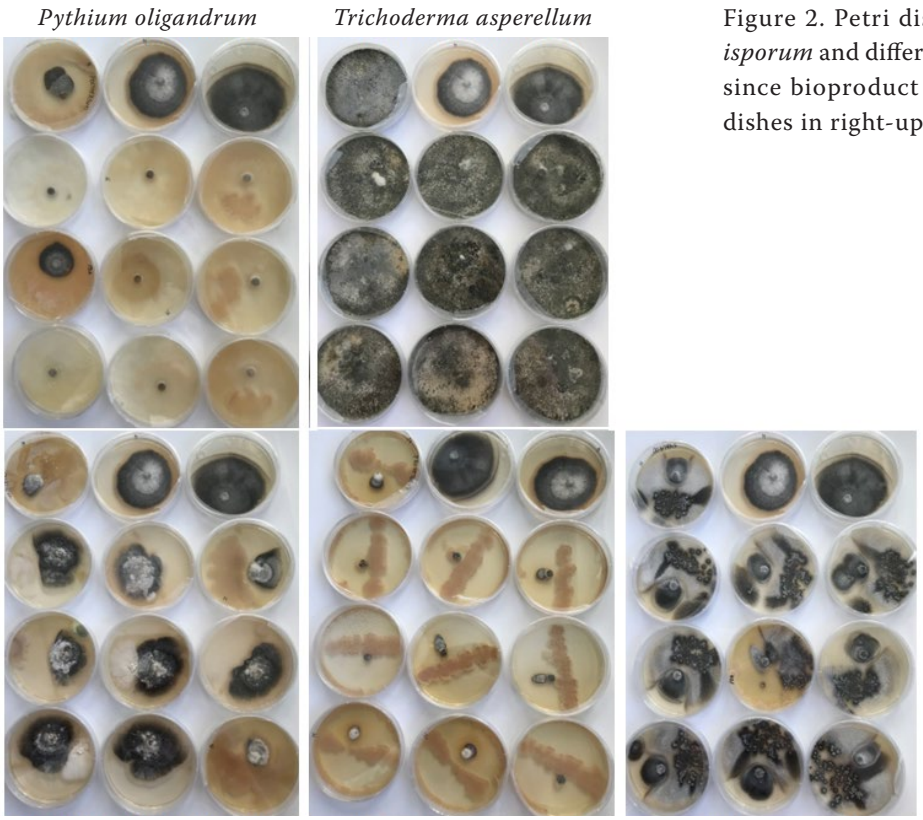

Figure 2. Petri dishes with *Verticillium longisporum* and different bioproducts after 6 weeks since bioproduct application (control 2 Petri dishes in right-up corner)

Dunker et al. (2008) hypothesised that soil and air temperatures may be important variables determining the colonisation of oilseed rape with the pathogen *Verticillium longisporum* in the field. Siebold and Tiedemann (2013) demonstrated a significant increase in *V. longisporum* colonisation of winter oilseed rape in a soil heating facility when soil temperatures were elevated by 1.6 or 3.2 °C concerning ambient temperature, indicating a higher vulnerability of spring-sown crops growing into the warmer season.

In field conditions, infections could already occur in autumn (Zeise and Seidel 1990); initiated by root exudates from the host plants, microsclerotia germinate, form hyphae that attach to the root surface and infect the plant (Mol 1995). The real-time PCR method can detect the fungus in the plant at an early stage and during its biotrophic life phase (Knüfer 2011). This fact was confirmed in our work and gives a good premise for future work, especially for a practical conventional use of this technique to determine *Verticillium longisporum* in plants.

Table 3. Amounts of *Verticillium longisporum* (μg in 0.1 g of plant material) on 3 localities (each repetition)

Variation	Chlumec nad Cidlinou			Trutnov			Kujavy		
Coniothyrium minitans	×	0.943	×	9.87	756	×	×	×	×
Pythium oligandrum 1	×	938	867	×	×	×	×	×	×
Pythium oligandrum 2	×	0.904	×	0.941	×	0.937	×	×	×
Pythium oligandrum 3	×	×	×	0.959	×	×	×	×	×
Pythium oligandrum 4	0.889	92.3	×		9.51	0.98	×	×	×
Trichoderma asperellum	9.04	×	×	9.59	×	×	×	×	×
Pseudomonas veronii	–	–	–	840	×	×	–	–	–
Bacillus amyloliquefaciens	0.929	×	×	–	–	–	0.904	×	×
Alicante (non-treated)	0.892	×	×	86.7	×	×	×	×	×

× – no pathogen presence detected

https://doi.org/10.17221/492/2023-PSE

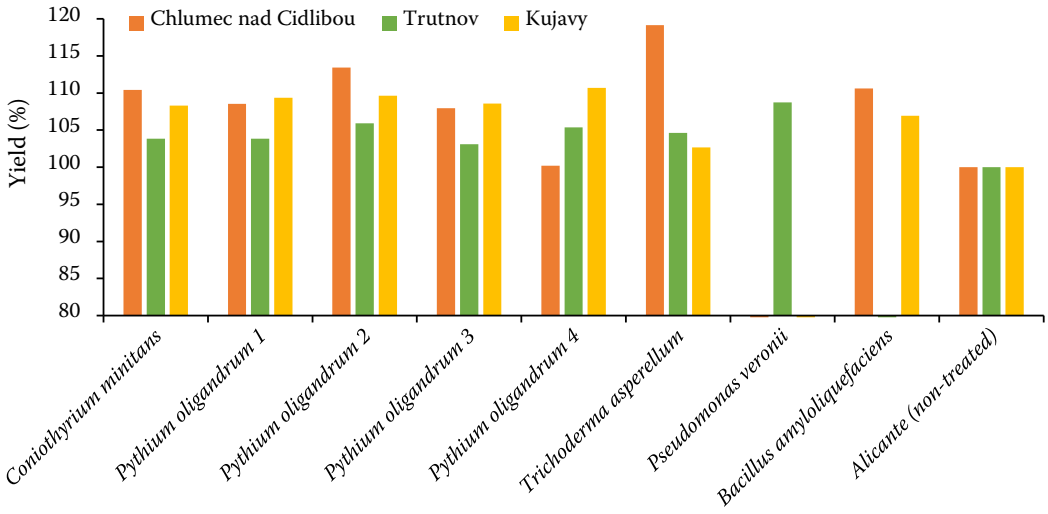

Figure 3. Yield expressed as a percentage of non-treated variation (100%) on 3 localities

REFERENCES

Barker K. (1998): Phenol-Chloroform Isoamyl Alcohol (PCI) DNA Extraction. Seoul, Hanyang University.

Bekker T.F., Kaiser C., v.d. Merwe R., Labuschagne N. (2006): *In-vitro* inhibition of mycelial growth of several phytopathogenic fungi by soluble potassium silicate. South African Journal of Plant and Soil, 23: 169–172.

Depotter J.R.L., Deketelaere S., Inderbitzin P., Von Tiedemann A., Höfte M., Subbarao K.V., Wood T.A., Thomma B.P.H.J. (2016): *Verticillium longisporum*, the invisible threat to oilseed rape and other brassicaceous plant hosts. Molecular Plant Pathology, 17: 1004–1016.

Dunker S., Keunecke H., Steinbach P., von Tiedemann A. (2008): Impact of *Verticillium longisporum* on yield and morphology of winter oilseed rape (*Brassica napus*) in relation to systemic spread in the plant. Journal of Phytopathology, 156: 698–707.

Eynck C., Koopmann B., Grunewaldt-Stöcker G., Karlovsky P., von Tiedemann A. (2007): Differential interactions of *Verticillium longisporum* and *Verticillium dahliae* with *Brassica napus* detected with molecular and histological techniques. European Journal of Plant Pathology, 118: 259–274.

Eynck C. (2008): Identification of resistance sources and characterization of resistance factors in *Brassica* species to *Verticillium longisporum*. Dissertation. Göttingen, University of Göttingen.

Heale J.B., Karapapa K.V. (1999): The *Verticillium* threat to Canada's major oilseed crop: canola. Canadian Journal of Plant Pathology, 21: 1–7.

Hornig H. (1987): Weitere Untersuchungen und Erkenntnisse zur Krankhaften Abreife. Raps, 1: 27–30.

Knüfer J. (2011): Improvement of winter oilseed rape resistance to *Verticillium longisporum* – assessment of field resistance and characterization of ultrastructural plant responses. Dissertation. Göttingen, University of Göttingen.

Mol L. (1995): Effect of plant roots on the germination of microsclerotia of *Verticillium dahliae*. II. Quantitative analysis of the luring effect of crops. European Journal of Plant Pathology, 101: 679–685.

Mol L., Riessen H.W. v. (1995): Effect of plant roots on the germination of microsclerotia of *Verticillium dahliae*. European Journal of Plant Pathology, 101: 673–678.

Olsson S., Nordbring-Hertz B. (1985): Microsclerotial germination of *Verticillium dahliae* as affected by rape rhizosphere. FEMS Microbiology Letters, 31: 293–299.

Schnathorst (1981): Life cycle and epidemiology of *Verticillium*. In: Mace M.E., Bell A.A., Beckman C.H. (eds.): Fungal wilt diseases of plants. London, Academic Press, 81–111.

Siebold M., Tiedemann A.V. (2013): Effects of experimental warming on fungal disease progress in oilseed rape. Global Change Biology, 19: 1736–1747.

Tenuta M. (2019): Pathogenic *Verticillium longisporum* found in Canada but yield loss is low, so far. Winnipeg, Canola Council of Canada.

Vega-Marin M., von Tiedemann A. (2022): Cross-protection of oilseed rape against *Verticillium longisporum* by the non-aggressive lineage A1/D2. BioControl, 67: 419–431.

Zeise K., Seidel D. (1990): Zur Entwicklung und Schadwirkung der *Verticillium* Welkekrankheit am Winterraps. Raps, 8: 20–22.

Zheng X., Lopisso D.T., Eseola A.B., Koopmann B., Von Tiedemann A. (2019): Potential for seed transmission of *Verticillium longisporum* in oilseed rape (*Brassica napus*). Plant Disease, 103: 8.

Zheng X., Koopmann B., Ulber B., Von Tiedemann A. (2020): A global survey on diseases and pests in oilseed rape – current challenges and innovative strategies of control. Frontiers in Agronomy, 2: 590908.

Zhou L., Hu Q., Johansson A., Dixelius C. (2006): *Verticillium longisporum* and *V. dahliae*: infection and disease in *Brassica napus*. Plant Pathology, 55: 137–144.

Received: December 16, 2023
Accepted: February 12, 2024
Published online: March 5, 2024