Microbial Preparations for Control and Prevention of Fungal Disease on Canola

Control of *Sclerotinia sclerotiorum* and *Leptosphaeria maculans* (*Phoma Lingam)*

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The active ingredient of biopreparation *Polyversum* is fungi-like organism *Pythium oligandrum.* The *Pythium oligandrum* colonizes the rhizosphere of treated plants. Because of its strong mycoparasitical and competitive abilities, suppress the growth and antagonistic effects of many soil-borne pathogenic fungi causing damping off and rots of roots and plants bottoms. The *Polyversum* also controls those canola diseases:

Alternaria black spot (*Alternaria brassicae*)

Blackleg of canola (*Leptosphaeria maculans)*

Sclerotinia Stem Rot (*Sclerotinia sclerotiorum*)

Gray Mold (*Botrytis cinerea*)

Pythium oligandrum also induces defense reaction in the plant, through stimulation of the phytohormones, which are involved in the resistance mechanisms of the plant against diseases. *Pythium oligandrum* does not produce any antibiotics and therefore isconsidered a true plant growth promoter for the induction of plant resistance. *Pythium oligandrum* has also significant growth stimulation effects which results into increased yield.

The effectiveness from trials done in the Czech Republic by accredited trial stations

In the listed tabs are summarized results from 10 trials from 5 trial stations in the run of three years. For maintaining data were chosen observing of 4 general diseases for winter canola and in lesser extent for summer canola.
Selected Diseases: *Alternaria brassicae*
Botrytis cinerea
Leptosphaeria maculans (Phoma lingam)
Sclerotinia sclerotiorum

Since, that was quite impossible to get as reference preparation other corresponding biological preparation registered for proposed way of use, were picked as referential chemical based preparations most widely used against the scale of pathogens in working conditions in the Czech Republic.

Selected chemical based referential preparations:
Fungicides:
Caramba - metconazole - spray dose 1.5 l/ha respective 1.2 l/ha
Horizon - tebuconazole - spray dose 1.5 l/ha
Rovral - iprodione - spray dose1.5 l/ha
Vitavax 200 FF - carboxim, thiram - seed dressing 4 l/t of seed

Growth stimulators

Atonik - 2- methoxy- natrium 5-nitrofenol - spray 0.6 l/ha
In the trials were used following ways of application of Polyversum:
Rate:
Dressing seeds 2kg/t
One spray 100 g/ha dissolved in 300-400 l of water
Two sprays 2 x 100 g/ha dissolved in 300-400 l of water
Three sprays 3 x 100 g/ha dissolved in 300-400 l of water
Researchers also tested sprays, when was Polyversum dissolved in liquid nitrogen fertilizer DAM 390.

The preparation POLYVERSUM demonstrated efficiency against all above-mentioned diseases in various scales. The efficiency of tested preparation is in congruency with the efficiency of chemical preparations. When Polyversum had lesser efficiency in special conditions, chemical fungicides did not work either.

Alternarias were observed in 9 trials, and in all cases the results of Polyversum were comparable witch chemical standard.

Botrytis was observed only in 5 trials and in 4 cases the results were comparable with chemical standards.

*Leptosphaeria maculan*s was observed in all of 12 trials. In 11 cases the results were comparable with standard chemical treatment in the same trial.

Sclerotinia was observed in 11 trials. In all cases the results of tested preparation were compared with chemical standards.

Phytotoxicity of tested preparation did not express in any variant of all trials, so the preparation can be considered as fully safe for treated plants.

On a basis of results of the submitted evaluations, two diseases of canola : *Leptosphaeria maculans* and *Sclerotinia sclerotiorum* were chosen for the application for approving of Polyversum..

***Leptosphaeria maculans* - Evaluation**
Entire data about trials

Legend to tab "*Leptosphaeria maculans*"

Application of POLYVERSUM

Seed dressing dry Seed dressing 2000g per ton of seed
1x spray 100g/ha
2x two sprays 100g /ha
3x three sprays 100g/ha
DAM 120 l of nitrogen liquid fertilizer per hectare in first treatment
Chemical treatment
VITA - seed dressing with Vitavax 200 FF - 4 l/t
Sprays:
ROV - Rovral 1.5 l/ha
CAR - Caramba 1.5 l/ha
HOR - Horizon 1.5 l/ha
Atonik 0.6 l /ha

	Growth stage of Canola (BBCH)	Date	Growth stage of Canola (BBCH)	Seed Dressing	1x	2x	3x	DAM	Ref. prep.	Atonik	% Efficiency	Stat. Cogency	Infestation
Domanínek	16.7.	17.4.	0	Yes							30,00	A %90,99	weak
2001	67	25.5.	13		Yes						20,00	A	
		8.6.	15			Yes					40,00	A	
median											30,00		
ref. prep.		17.4.	0						VITA		20,00	A	
Kujavy													
2001	16.8	17.4.		Yes							8,96	A %90,99	weak
	65-67	23.5.	13	Yes	Yes						20,90	A	
		7.6	39-42	Yes		Yes					23,88	A	
median											17,13		
ref. prep.		17. IV.							ROV		20,90	A	
Krásné Údolí	20.7.	25.4.		Yes							48,50	B %90,99	medium
2001	67-69	31.5.	13	Yes	Yes						60,80	BC	
		15.6.	19	Yes		Yes					69,44	C	
median											59,58		
ref. prep.									CAR		61,79	BC	
	26.8.			Yes							46,24	B %90,99	medium
	83			Yes	Yes						55,16	B	
				Yes		Yes					54,46	B	
median											51,95		
ref. prep.									CAR		48,36	B	
sum.				Yes							47,19	B, B	medium
				Yes	Yes						57,48	C, BC	%95,99
				Yes		Yes					60,67	C, C	
median											55,35		
ref. prep.									CAR		53,96	BC, BC	

Comments:

Testing of preparation POLYVERSUM was done in 10 trials a preparation was compared with fungicides Rovral, Caramba, Horizon, Vitavax 200 FF. Mentioned preparations were applied to winter and summer canola in combination with plant growth stimulator Atonik as states in tabs.
Polyversum was applied 48 times.
In 30 cases was reached better evaluation than by reference fungicide treatment.
In 7 cases was efficiency of Polyversum higher than 50 %, but lower than efficiency of chemical preparations.
In 11 cases were results of tested biopreparation worst than chemical standard.

The preparation was applied in one, two or three terms growth stages of canola.
First treatment in stages (BBCH) 18 -25
Second treatment in stages (BBCH) 30 - 38
Third treatment in stages (BBCH) 45 60
Application is fully in correspondence with recommendation of manufacturers of referential fungicidal standards. (BBCH 11-32 and 32- 60)

Infection ascends in time of observations from low to medium pressure.

Gained values are seemingly non-homogenous. That is reason why a correlation coefficient between group treated with chemical fungicides and between group treated with Polyversum was calculated. The value of this coefficient being 0.886, the correlation between both groups was very tight.
(Tab value for a 0.005 16 terms is 0.6622). Low values in both groups were caused by other events than for tested preparation. This fact confirmed contradictory phenomena. High efficiency in both assessed files in the same locality. These phenomena - high or low efficiency- were identically influenced either by force of infection, climatic conditions in specific locality or condition of the crop.

In the case of occurrence and development of pathogen, both groups showed high efficiency.

Above results show that efficiency of preparation Polyversum is comparable with chemical standards- this is documented by statistical cogency and is influenced by instantaneous condition of environment as well as artificial fungicides.

The best method of application seems to be two or three sprays.
First treatment in stages (BBCH) 25 30
Second treatment in stages (BBCH) 35 45
Third treatment in stages (BBCH) 45 - 55

The application with liquid fertilizer DAM 390 is possible.

For practical application is suitable to modify application window as is showed:

Treatment	Growth stage of canola
1	BBCH 15 - 30
2	BBCH 30 - 40
3	BBCH 40 - 65

***Sclerotinia sclerotiorum* - Evaluation**

Entire data about trials

	Growth stage of Canola (BBCH)	Date	Growth stage of Canola (BBCH)	Seed Dressing	1x	2x	3x	DAM	Ref. prep.	Atonik	% Efficiency	Stat. Cogency	Infestation
Domanínek	20.VII	25.4.	34		/						40,58	AB %90	medium
2001	85	9.5.	61			/					56,52	B	
		9.5.	61			/		/			62,32	B	
median											53,14		
ref. prep.		25.4.	34						ROV	/	38,55	AB	
2001 Kujavy	11.7.	6.4.	30-32		/			/			18,95	A	low
	83-85	24.4.	50-51			/		/			7,37	A	
		24.4.	50-51			/		/			4,21	A	
median											10,18		
ref. prep.		9.5.	63-65						ROV	/	10,53	A	
Krásné Údolí	24.7.	27.4.	30		/						51,04	AB %99	medium
2001	85	8.5.	32			/					55,76	B	
		8.5.	32			/					54,25	B	
median											53,68		
ref. prep.		27.4.	30						HOR	/	55,09	B	
Nechanice	3.7.	17.4.	34			/				/	45,55	B, AB	95,99%
2002	81	30.4.	60			/		/			46,32	B, AB	medium
		24.4.	50							/	51,69	B, B	
median											47,85		
ref. prep.		17.4.	34						CAR		16,41	AB, AB	
Opava	9.7.	9.4.	33			/					16,67	A %90,99	weak
2002	81	16.4.	51-52			/		/			55,56	A	
		23.4.	53-57				/				41,67	A	
median											37,97		
ref. prep.		9.4.	33								5,56	A	
									CAR	/			
Krásné Údolí	4.6.	21.4.	30			/					30,18	AB, A %90	95,90%
2002	67	4.5.	31			/					58,04	B, A	medium
		28.5.	51				/				33,13	AB, A	
median											40,45		
ref. prep.		21.4.	30						CAR	/	28,45	A, A	
	15.6.					/					44,37	B	%90-99
	75					/					47,78	B	medium
							/				49,49	B	
median											47,27		
ref. prep.									CAR	/	39,59	B	

	Growth stage of Canola (BBCH)	Date	Growth stage of Canola (BBCH)	Seed Dressing	1x	2x	3x	DAM	Ref. prep.	Atonik	% Efficiency	Stat. Cogency	Infestation
Domanínek	15.7.												
2002	8.7.	17.4.	30			/					16,33	AB, A	medium
		26.4.	39			/		/			25,50	B, A	%90-95,99
		7.5	60				/				27,34	B, A	
median											23,06		
ref. prep.		26.4.	39						CAR	/	18,35	AB, A	
Krásné Údolí	6.8.	5.11.	14			/					32,86	B	medium
2004	83	22.4.	30			/					34,51	B	
		30.4.	39				/				39,69	B	
median											35,67		
ref. prep.		30.4.							CAR		36,87	B	
Nechanice	18.7.	30.9.	15			/					35,78	B	weak
2004	85	9.4.	33			/					35,22	B	
		23.4.	53				/				43,12	B	
median											38,04		
ref. prep.		9.4.							CAR		40,37	B	

	Growth stage of Canola (BBCH)	Date	Growth stage of Canola (BBCH)	Seed Dressing	1x	2x	3x	DAM	Ref. prep.	Atonik	% Efficiency	Stat. Cogency	Infestation
Domanínek	5.9.	17.4.	0	/							-13,04	A % 90,99	week
2001	85	25.5.	13		/						47,83	A	
		8.6.	15			/					30,43	A	
median											21,74		
ref. prep.		17.4.	0						VITA		0	A	
Krásné Údolí	28.8.	25.4.		/							39,33	B, B, B	medium
2001	83-85	31.5.	13	/	/						42,37	BC, BC, B	% 90,95,99
		15.6.	19	/		/					58,06	C, C, B	
median											46,59		
ref. prep.		31.5.							CAR		55,87	BC, C, B	

Legend to tab "*Sclerotinia sclerotiorum*"

Application of POLYVERSUM

Seed dressing dry Seed dressing 2000g per ton of seed
1x spray 100g/ha
2x two sprays 100g /ha
3x three sprays 100g/ha

DAM 120 l of nitrogen liquid fertilizer per hectare in first treatment

Chemical treatment

VITA - seed dressing with Vitavax 200 FF - 4 l/t
Sprays:
ROV - Rovral 1.5 l/ha
CAR - Caramba 1.5 l/ha
HOR - Horizon 1.5 l/ha
Atonik 0.6 l /ha

Values written in RED are higher than the referenced chemical

Values written in GREEN are lower than the referenced chemical.

Values writtenin BLUE are lower than the referenced chemical, but with efficiency increased by 50%

Values written in PURPLE indicate the efficiency of the referenced preparation.

Commentary:

A preparation of POLYVERSUM was tested and compared in 9 trials with preparations of the chemical fungicides Rovral, Caramba, Horizon and Vitavax 200 FF.
All of the preparations were applied to winter and summer canola in combination with the plant growth stimulator Atonik as stated in the tabs.
Applications of POLYVERSUM were applied 30 times:
in 23 cases, POLYVERSUM tested better than the chemical treatments;
in 2 cases, the efficiency of POLYVERSUM was higher than 50% of, but lower than the efficiency of the chemical treatments; in 6 cases POLYVERSUM tested lower than the chemical standard.
Applications of each of the prepaparations were carried out in the following growth stages of the crops:
BBCH 18-25, for summer canola; or BBCH 30-52, for winter canola.

Application was in full compliance with the each of the manufacturers recommended fungicidal standards (BCH 18-25 and 30-52).Infection ascends in time of observation from low to medium pressure.

Observed values are seemingly non-homogenous. For this reason, the correlation coefficient between groups treated with chemical fungicides and groups treated with POLYVERSUM are calculated separately. The coefficient for POLYVERSUM for 10 terms is 0.7143 and its correlation with the chemical teatment is very tight (Tab value for a 0.025 - 10 terms is 0.6319).

Low values in both groups are caused by events other than differences in the preparations themselves, confirming contradictory phenomena -high efficiency in both assessed files in the same locality. These phenomena - high or low efficiency are identically influenced either by force of infection, climatic conditions in a specific locality or condition of the crop. In cases of occurrence and development of pathogen, both groups show high efficiency.

From the above results, the efficiency of the preparation POLYVERSUM is comparable with chemical standards - as documented by statistical cogency - and is influenced by the instantaneous state and condition of the environment to the same extent as artificial fungicides

The best method of application appears to be two or three sprays:
First treatment in stages (BBCH) 24 - 30
Second treatmentin stages (BBCH) 35 -45
Third treatment in stages (BBCH) 45 - 55

Application with liquid fertilizer DAM 390 is acceptable.

For practical application, the application window may be modified as shown:

Treatment	Growth stage of canola
1	BBCH 15 - 30
2	BBCH 15 - 30
3	BBCH 15 - 30

Statistical comparison efficiencie of Polyversum and standards.

Calculation of correlation between medians of Polyversum treatments and chemical standards

Sclerotinia sclerotiorum			
		Efficiency %	
Year	Locality	Median Polyversum	Chemical Treatment
2001 winter can.	Domanínek	53,14	38,55
2001 winter can.	Kujavy	10,19	10,63
2001 winter can.	Krásné Údolí	53,08	55,09
2002 winter can.	Nechranice	47,85	16,41
2002 winter can.	Opava	37,97	5,56
2002 winter can.	Krásné Údolí	40,45	28,45
2002 winter can.	Domanínek	47,21	39,59
2004 winter can.	Krásné Údolí	35,87	36,37
2004 winter can.	Nechanice	33,10	40,37
2002 summer can	Domanínek	23,00	18,35
2001 summer can	Krásné Údolí	45,69	55,87
	Sclerotinia	r = 0,7143	tab 0,025/10 = 0,6319
			0,05/10 = 0,5493
			0,05/10 = 0,7155

Leptospaeria maculans (Phoma)			
		Efficency %	
Year	Locality	Median Polyversum	Chemica Treatment
2001 winter c.	Domanínek	25,29	19,53
2001 winter c.	Kujavy	16,87	10,00
2001 winter c.	Krásné Údolí	49,49	38,01
	Strong infestation	60,29	62,15
	Medium infestation	62,11	70,50
2002 winter c.	Nechranice	60,73	56,12
2002 winter c.	Opava root collar	10,79	14,20
	Whole plant	9,29	3,23
2002 winter c.	Krásné Údolí 1st	20,01	10,02
	2nd	54,42	71,77
2002 winter c.	Domanínek	13,33	19,13
2004 winter c.	Krásné Údolí	15,72	14,45
		19,78	19,13
		4,36	3,74
2004 summer c.	Nechanice	58,24	66,67
2001 summer c.	Domanínek	30,00	20,00
2001 summer c.	Kujavy	17,13	20,90
2001 summer c.	Krásné Údolí	59,98	61,79
	2nd	51,95	48,33
	sum	55,35	53,93
	Phoma	r = 0,6975	tab 0,005/16 = 0,6622
			0,01/13 = 0,59

0,01/16 = 0,59

Treatments:
Seed Treatment:
Seed can be dressed in dressing machines.The dose is; 2 g. per kg. of seed

Treatments:

Three sprays are recommended for winter canola : the first treatment is recommended in autumn, 14 days after plant emeregence ; the second treatment is recommended in the spring, when the temperature is over 10 C° ; the third treatment should follow 2 - 3 weeks later. Two sprays are recommended for summer canola.

The first spray is recommended in the month following plant emergence; the second treatment should follow 2-3 weeks later.

The spray volume is 100g. of Polyversum per hectare (10,000 m2) in minimum amount of 400L water.

Mix Tank Recommendations: Mix dose for sprayer tank with water in small vessel (15 L bucket); stir well.

Then fill mixing tank one-half full of water, pass content of mixing vessel through fine sieve into tank and add remaining water to sprayer. The preparation can be mixed, then sprayed together with liquid fertilizers, herbicides and insecticides.

Because in both cases (*Leptosphaeria maculans, Sclerotinia sclerotiorum)* the efficiency of Polyversuim exceeded on average the efficiency of chemical standards, we can discuss Polyvesum as comparable to the referential preparations.

[illegible]

Variant	*Type*	*Used preparations*
K	Non-treated control	
CH	Referential preparations	9.4.2002 2-methoxy-5-nitrofenol 0,6 l/ha + meteonázole 1,2 l/ha + water 400 l/ha
		23.4.2002 2-methoxy-5 nitrofenol 0,6 l/ha + water 400 l/ha
P 2	testing	9.4.2002 Polyversum 100 g/ha + water 400 l/ha 6.4.2002 - Polyversum 100 g/ha + water 400 l/ha
P 2 + DAM	testing	9.4.2002 - Polyversum 100 g/ha + water 400 l/ha
		16.4.2002 - Polyversum 100 g/ha + 20 l of liquid fertilizer DAM/ha
		9.4.2002 - Polyversum 100 g/ha + water 400 l/ha
P 3	testing	16.4.2002 - Polyversum 100 g/ha + water 400 l/ha
		23.4.2002 - Polyversum 100 g/ha + water 400 l/ha

Winter canola variety Zorro
Trial station Nechanice, district Hradec Králové

Varinat	*Type*	*Used preparations*
K	Non-treated control	
CH	Referential preparations	17.4.2002 - 2-methoxy- 5-nitrofenol Na 0,6 l/ha – meteonazole 1,5 l/ha + water 400 l/hc
P 2	testing	17.4.2002 - Polyversum 0,1 kg/ha + water 400 l/ha
		30.4.2002 - Polyversum 0,1 kg/ha + water 400 l/ha
P 2 + DAM	testing	17.4.2002 - Polyversum 0,1 kg/ha + water 400 l/ha
		30.4.2002 Polyversum 0,1 kg/ha + 120 l of liquid fertilizer DAM/ha
P 3	testing	17.4.2002 - Polyversum 0,1 kg/ha + water 400 l/ha
		24.4.2002 - Polyversum 0,1 kg/ha + water 400 l/ha
		30.4.2002 - Polyversum 0,1 kg/ha + water 400 l/ha

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