

**Research Article**

**The Influence of Spring Barley Extracts On  
*PSEUDOMONAS PUTIDA* PCL1760**

**Safin R.I., Validov Sh.Z., Karimova L.Z.,  
Karimov Kh.Z., Safiollin F.N. and Minnullin G.S.**

Kazan State Agrarian University

[Received: 05/12/2018; Accepted: 14/01/2019; Published: 15/01/2019]

**ABSTRACT.**

**Background.** Biocontrol microorganisms contained in biopreparations that are used for seed dressing are prone to the influence of external factors, including those substances that are expressed by the plants during germination. This influence can differ depending on the content of the expressed substances. Different extracts, obtained from germinating seeds, can be used for the study and modeling of this influence. The study of such extracts will allow the scientists to develop new preparations for enhancement of biofungicide bioagents activity and plant growth stimulation.

The aim of the present study was to evaluate the influence of different extracts (water, ethanol, enzymatic) from germinated seeds of spring barley on the development of *Pseudomonas putida* bacteria.

**Scope.** The tests on optical density were performed to study the influence of different extracts on the development of model extracts.

The analysis of the influence of different extracts from germinated seeds of spring barley on biological agent - *Pseudomonas putida* PCL1760, contained in biopreparations for crop protection, showed that ethanol extract from Raushan spring barley increased the resistance of *Pseudomonas putida* PCL1760 to the heat and osmotic stresses. All the types of extracts did not contribute to the increase of bacteria resistance to low temperatures. There was no significant difference established between the activity of spring barley extracts with vernalization and without vernalization.

**Conclusion.** The materials of the study showed that ethanol extracts from spring barley can be used for the development of *Pseudomonas spp.* based biopreparations for the enhancement of biocontrol microorganisms (BCMs) activity.

**Key words:** biocontrol microorganisms, microorganism stress, *Pseudomonas putida*, plant extracts, spring barley.

**INTRODUCTION**

One of the most important approaches to crop protection from the diseases is the application of biofungicides. Their activity is determined by their biological agent (Janisiewicz and Korsten et al., 2002; Chaube et al., 2003; Islam et al., 2005; Singh et al., 2012). When the seeds are treated with biofungicides, the biocontrol microorganism is prone to the influence of different chemical compounds. Seed

germination is characterized by a pool of compounds (exudates); many of them exert increased biological activity, including antimicrobial (Randy et al., 2009; Dutta and Podile, 2010; Ling et al., 2011). The influence of such exudates on the activity of biological agents is understudied. To study the activity of exudates on biofungicide bioagents, it is necessary to distribute these compounds into

groups, because the study of the influence of separate compounds limits the possibilities of the evaluation. The issue can be solved by the application of different seeds extracts.

Currently, plant-derived physiologically active substances, obtained by different extraction methods, attract researchers' interest (Zhang et al., 2008; Khan et al., 2009). In particular, amino acids and proteins, obtained during plant extraction, show significant growth stimulation effect during application for different agricultural crops (Calvo et. al., 2014). The extracts exert significant positive effect on the nitrogen consumption, optimization of C:N ratio as well as plant hormonal status (Colla et. al., 2014). Protein hydrolysates stimulate the growth and development of different microorganisms, including soil ones (du Jardin, 2016).

Seed extracts attract researchers' attention because extracts preparations from seed material is quick and replicable, exudates from the germinating seeds and roots are quite similar (Vancura & Hanzlikova, 1972), and (iii) effective interaction between a number of biocontrol microorganisms and plants is observed at the stage of seed germination (Jacoud et al., 1998).

Different methods are used for extraction of plant-derived physiologically active substances. The most widespread methods are water, ethanol and enzymatic extraction (Pothier et al., 2007). The composition of an extract changes depending on the method of extraction. In particular, hot water is used primarily for extraction of water-soluble proteins (albumins, etc.), ethanol – for alcohol-soluble proteins (prolamins, etc.), and enzymes – for different amino acids. Plants can be used as a source of material for extracts that enhance BCMs resistance to stress. However, particular interest is drawn to spring barley.

## MATERIALS AND METHODS

The study was conducted at the Department of General farming, crop protection and breeding at the Kazan State Agricultural University.

Raushan spring barley (*Hordeum distichon* L.) was used for extracts preparation. Before germination, the seeds were sterilized in 70%

ethanol for 5 minutes. After drying out in sterile filter paper, the seeds (100 pcs x 3 replications) were placed in moist chambers (plant culturing units with sterile filter paper and sterile water). Germination was performed by two methods. The first method: germination was performed at +24°C (without vernalization) for 7 days. The second method: germination was performed at +24°C for 3 days and then the seeds were placed in a cooling chamber at + 8°C for 3 days (with vernalization). Seven days after, the seeds were dried out to 14% of moisture and milled in a lab mill. As a result, milled crop for extraction was obtained. Fined air-dried dark grey forest soil (humus content 5.2%) was used as a standard sample.

**Extraction.** 1 g of the studies material was used for each extraction.

**Water extract:** 85°C 10 ml of sterile distilled water was added to 1 g of the material. Time of extraction was 12 hours. After the extraction, supernatant fluid was filtered through a paper filter. 5 ml of extract was added sterile water to the volume of 10 ml. All the tests were replicated three times.

**Ethanol extract:** 25°C 10 ml of 95% ethanol was added to 1 g of the material. The time of extraction was 12 hours. After the extraction, supernatant fluid was filtered through paper filter. Ethanol was evaporated and the residue was diluted in 10 ml of sterile water.

**Enzymatic extract:** 1 g of the material was added 1 mg of pancreatin (digestive enzyme, 10 000 UA) and 35°C 10 ml of distilled water. The time of extraction was 12 hours. After the extraction, supernatant fluid was filtered through a paper filter. 5 ml of extract was added distilled water to 10 ml. All the tests were replicated three times.

**Tests variants:** 1. water extract without vernalization - WEWV; 2. ethanol extract without vernalization – EEWV; 3. enzyme extract without vernalization – EnEWV; 4. water extract with vernalization – WEV; 5. ethanol extract with vernalization – EEV; 6. enzyme extract with vernalization – EnEV; 7. soil water extract – SWE; 8. soil ethanol extract – SEE; 9. soil Enzyme extract – SEnE.

**Evaluation of the extracts activity towards***Pseudomonas putida*

The seeds were germinated in the growing medium BM containing 7.6 g/L  $K_2HPO_4 \cdot 3 H_2O$ , 3 g/L  $KH_2PO_4$ , 1 g/L  $(NH_4)_2SO_4$  and 0.2 g/L  $MgSO_4 \cdot 7 H_2O$  with or without 2% glucose solution. This medium is a modified succinate Meyer medium (Meyer and Abdallah, 1978)

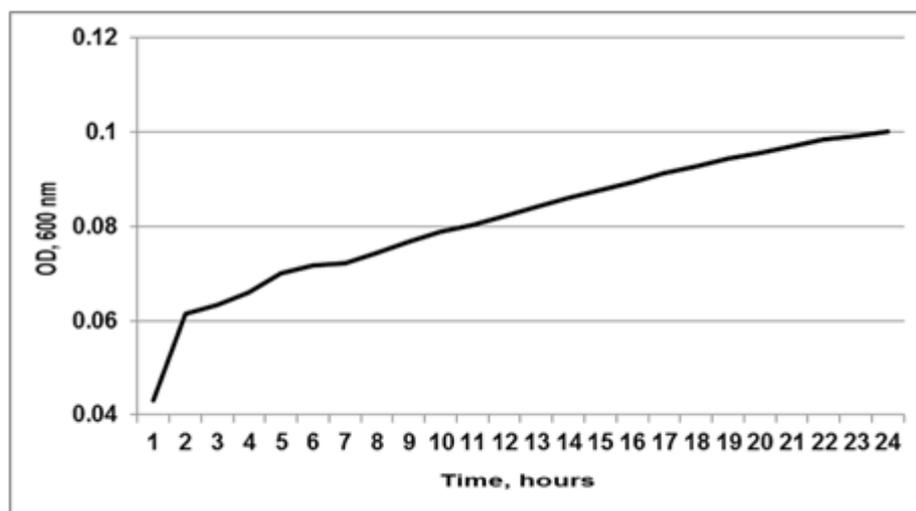
*Pseudomonas putida* PCL1760 strain was used for the tests on the evaluation of plant extracts influence on microorganisms. This strain was isolated from the rhizosphere of avocado. This bacterium is an active colonizer of plant rhizosphere (Validov et al. 2007). Besides, PCL1760 is a microorganism for biological control of tomato root rot. It exerts protective function by competing for ecological niches (Validov, 2007). The strain *P. putida* PCL1760 is a prototrophe, i.e. it grows in mineral media and its optimal temperature for growth is 30°C. To evaluate its influence on PCL1760 strain, 50 µL of each extract was added to 150 µL of BM medium already mixed with a suspension of

PCL1760 strain cells to the concentration of ( $OD_{600} = 0.01$ ), which corresponds to  $10^7$  cells/ml. Each extract was tested in three wells of the plate for statistical processing of the data. The plates were incubated at 30 °C for evaluation of the extracts influence on the growth of PCL1760, and for evaluation of the extracts influence under salt, osmotic and cold stress.

For evaluation of extracts influence on the strain PCL1760 under the heat stress, the plates were incubated under 37°C. Optical density of the culture was measured with iMark (Bio-Rad Laboratories, USA) at wavelength 600 nm.

**RESULTS.**

To define the test parameters, the authors obtained the growth curve for *P. putida* PCL1760 strain in the growing medium BM containing glucose (Fig. 1). After inoculation with the rated 0.01, the culture achieved the density of 0.1 ( $OD_{600}$ ), which corresponded to the density of  $10^8$  cells/ml of culture.



The results of the extracts influence in the growth of *P. putida* bacteria in minimum essential medium BM are presented in Table 1.

**Table 1.** Influence of extracts on the development of *P. putida* PCL1760 in the minimum essential medium BM without glucose (optical density at 600 nm), 2017

Variant	1 <sup>st</sup> series of tests	2 <sup>nd</sup> series of tests	Mean	Significance of difference to control
Water - control				
WEWV	0.056	0.066	0.061	–
EEWV	0.294*	0.163*	<b>0.229*</b>	+
EnEWV	0.073	0.067	0.070	–
WEV	0.080	0.082	0.081	–
EEV	0.098*	0.094*	<b>0.096*</b>	+

EnEV	0.074	0.071	0.073	–
SWE	0.051	0.052	0.052	–
SEE	0.075	0.076	0.076	–
SEnE	0.052	0.056	0.054	–

Note: \* – the difference as compared to the control is significant at P=0.05

Strong stimulating effect on the development of *P. putida* in the growing medium without glucose was exerted by ethanol extract without vernalization and with vernalization. Water and enzymatic extracts did not influence on optical density, as well as all the other soil extracts.

**Table 2.** Influence of extracts on the development of *P. putida* PCL1760 in the minimum essential medium BM without glucose (optical density at 600 nm, T=30° C), 2017.

Variant	1 <sup>st</sup> series of tests	2 <sup>nd</sup> series of tests	Mean	Significance of difference to control
Water - control	0.104	0.103	0.103	
WEWV	0.104	0.063*	0.083	–
EEWV	0.136*	0.126*	<b>0.131*</b>	+
EnEWV	0.071*	0.057*	0.064	–
WEV	0.102	0.074*	0.088	–
EEV	0.118	0.149*	<b>0.134*</b>	+
EnEV	0.091	0.094	0.093	–
SWE	0.064*	0.070*	0.067*	+
SEE	0.202*	0.134*	<b>0.168*</b>	+
SEnE	0.092	0.100	0.096	–

Note: \* – the difference as compared to the control is significant at P=0.05

Soil and spring barley seeds ethanol extract positively influenced on the development of *P. putida* in the medium with glucose. Water and enzymatic extracts did not influence on this parameter.

Cold stress test results brought some interesting results (Table 3).

**Table 3.** Influence of the extracts on the development of *P. putida* PCL1760 in the minimum essential medium BM with glucose (optical density at 600 nm, T=-20° C), 2017.

Variant	1 <sup>st</sup> series of tests	2 <sup>nd</sup> series of tests	Mean	Significance of difference to control
Water - control	0.076	0.087	0.082	
WEWV	0.064	0.066	0.065	–
EEWV	0.094	0.110	0.102	–
EnEWV	0.081	0.088	0.085	–
WEV	0.075	0.073	0.074	–
EEV	0.087	0.092	0.089	–
EnEV	0.087	0.092	0.090	–
SWE	0.066	0.072	0.069	–
SEE	0.101*	0.130*	<b>0.115*</b>	+
SEnE	0.065	0.099	0.082	–

Note: \* – the difference as compared to the control is significant at P=0.05

Positive changes in parameters of optical density were observed only when soil ethanol extracts were used, while in other test variants no significant difference was observed as compared to the control.

To model the influence of the heat stress, *P. putida* was cultivated at + 37° C (Table 4).

**Table 4.** Influence of the extracts on the development of *P. putida* PCL1760 in the minimum essential medium BM with glucose (optical density at 600 nm, T=+37° C), 2017.

Variant	1 <sup>st</sup> series of tests	2 <sup>nd</sup> series of tests	Mean	Significance of difference to control
Water - control	0.072	0.078	0.075	
WEWV	0.084	0.063	0.073	–
EEWV	0.096	0.180*	<b>0.138*</b>	+

EnEWV	0.071	0.063	0.067	–
WEV	0.072	0.074	0.073	–
EEV	0.078	0.167*	<b>0.122*</b>	+
EnEV	0.081	0.088	0.085	–
SWE	0.064	0.064	0.064	–
SEE	0.103	0.134*	<b>0.119*</b>	+
SEnE	0.066	0.075	0.071	–

Note: \* – the difference as compared to the control is significant at P=0.05

Under the conditions of the heat stress, spring barley seeds ethanol extracts had significant positive effect on the growth of *P. putida*. There was no significant difference between barley seeds extracts without vernalization and with vernalization.

The results of the tests on the extracts influence on the development of the bacteria under osmotic stress are shown in Table 5.

**Table 5.** Influence of the extracts on the development of *P. putida* PCL1760 in the minimum essential medium BM in the presence of glucose (optical density at 600 nm, T=+30° C, 3% solution of NaCl), 2017.

Variant	1 <sup>st</sup> series of tests	2 <sup>nd</sup> series of tests	Mean	Significance of difference to control
Water - control	0.081	0.081	0.081	
WEWV	0.083	0.119*	0.101	–
EEWV	0.133*	0.206*	<b>0.170*</b>	+
EnEWV	0.100	0.118*	0.109	–
WEV	0.102	0.164*	<b>0.133*</b>	+
EEV	0.090	0.138*	<b>0.114*</b>	+
EnEV	0.122*	0.140*	<b>0.131*</b>	+
SWE	0.080	0.108	0.094	–
SEE	0.125*	0.154*	<b>0.139*</b>	+
SEnE	0.064	0.104	0.084	–

Maximum values of the optical density parameter were observed in the variant with spring barley seeds extract without vernalization. Positive results were obtained in the tests of all the seeds extracts with vernalization and soil ethanol extracts.

## DISCUSSION.

The present study showed that the influence of different groups of extracts on biological agent *P. putida* PCL1760 is diverse. The most significant positive effect had ethanol extracts, which is explained by the presence of high concentrations of amino acid proline that is a well-known protectors of microorganisms from the stress impact under stress conditions (Selivanova, 2012). It can be suggested that the expression of this amino acid by the germinating seeds allows for biological agent activity

maintenance under conditions of abiotic stresses. The observed effect can be used as a basis for the development of specialized adaptogene drugs for the maintenance of biofungicide activity under unfavorable conditions. The development of this direction will allow the producers to increase the effectiveness of crops bioprotection from the diseases.

## CONCLUSION.

Raushan spring barley seeds ethanol extracts have positive effect on the increase of *P. putida* PCL1760 resistance to heat and osmotic stresses, but do not influence on the resistance to low temperatures. No differences were observed between the anti-stress activity of ethanol seed extracts without vernalization and with vernalization.

## RECOMMENDATIONS.

Application of ethanol extracts from germinated spring barley seeds can significantly increase the resistance of biocontrol microorganisms (BCMs) contained in biopesticides to the influence of unfavorable abiotic factors.

## ACKNOWLEDGEMENTS.

The present study was conducted with the financial support of the Ministry of Education and Science of the Russian Federation. The agreement for the subsidies - №14.610.21.0017. Unique identification code of the project - RFMEFI61017X0017.

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