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Innovative methods of seed refinement and increasing onion plant health and productivity by using microbiological consortiums enriched with natural compounds

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A b s t r a c t. The biggest problems limiting organic production is the heavy contamination of seeds by phytopathogens, which then spread to plants and cause a reduction in their yield or death. The aim of the research was to develop an innovative technology enabling improvement of seed quality and plant productivity by using beneficial microorganisms and biological agents. The organically produced commercial seeds were treated with whey, Skotan yeast, water suspensions of two consortiums of bacteria, M1 (*Pantoea* sp. X58AD and *Pseudomonas* sp. Ps118AA) or M2 *(Enterobacter* sp. Pi119AC and *Pantoea* sp. Pi72ED) isolated from the onion root rhizosphere, commercial microbiological biopreparations (Serenade, Contans, Polyversum, Totalhumus), and hot water (50°C). The organic seeds were strongly infected by saprotrophic and pathogenic fungi. All the seed treatments significantly reduced the occurrence of phytopathogens and increased seed germination kinetics and plant emergence, plant growth and bulb yields, gas exchange, and the index of chlorophyll content in leaves. The single seed treatments with the isolated bacterial consortium exhibited similar (M1) and higher (M2) effectiveness than the commercial preparations, and all of them had greater fungicidal effects than whey and the hydro-thermotherapy. The enrichment of isolated bacterial consortium M2 with whey and Skotan yeast and M1 with Totalhumus increased the fungicidal and growth stimulating effectiveness the most.

K e y w o r d s: onion, plant growth, germination, seed health, biological agents, antagonistic bacteria

1. INTRODUCTION

The most important problems limiting ecological plant production is the low quality of organically produced seeds and their strong infection by phytopathogens. Heavily infected seed material is a serious threat and a source of infection for future plants, which results in yield losses increasing year by year and in lowering the quality of reproduced seeds (Dorna *et al*., 2005; Medić-Pap *et al.*, 2022). Therefore, the use of high-quality seeds in ecological production of economically important plants becomes a priority, including onions of which the production in 2022 was 6 314 000 t in the EU countries and 644 100 t in Poland (Behr and Illert, 2022). Since most of the pathogens causing infectious onion diseases are transmitted through seeds, limiting their germination and plant development, their microbiological and sowing quality should be improved by applying pre-sowing treatments (Dorna *et al.,* 2005; Fernández *et al.,* 2011; Janas *et al.,* 2019; Medić-Pap *et al.,* 2022).

The current techniques of seed protection in organic systems are still not satisfactory and consist in using extracts from garlic, tea tree, tansy, field horsetail, and nettle, which protect plants against the development of pathogens. They also involve pre-sowing seed treatments with

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natural agents (yeast, pepper, turmeric, algae), biotechnical preparations, including humic compounds (Apolhumus, Totalhumus, Huminpol), and microbiological preparations containing antagonistic and parasitic fungi (Janas *et al.,* 2019). Recently, more attention has been paid to the use of antagonistic and parasitic microflora cells isolated from the rhizosphere soil and roots of different plants, with the most extensive research in this area devoted to the use of *Trichoderma harzianum, T. harzianum Rifai* KRL-AG2, *T. harzianum* 3013, and *Stachybotrys chartarum* (Hanci *et al*., 2014; Abo-Elyousr *et al*., 2017), and a mixture of microorganisms and natural remedies, *e.g*. fish wastes or mycorrhizal arbuscular fungi that may have different modes of action or may provide synergistic effects (Bennett *et al.,* 2009; Metwally *et al*., 2021; Abdelhameed and Metwally, 2022; Cardarelli *et al.*, 2022).

The assumption of the presented research is that the combined use of a mixture of selected native microorganisms (*Pseudomonas, Pantoea,* and *Enterobacter* isolates from onion root rhizosphere), natural remedies, and biotechnical preparations having a biocidal effect should be more effective in decreasing pathogen infestation than single biological agents used to date. To the best of our knowledge, no research using the aforementioned mixtures of native selected antagonistic bacteria, natural remedies, and biopreparations in organic onion crops has been conducted in this area.

The aim of the present study was to examine the effects of seed treatment with whey, Skotan yeast, water suspensions of isolates from the rhizosphere of onion roots: M1 (*Pseudomonas* sp. Ps118AA and *Pantoea* sp. X58AD) and M2 (*Enterobacter* sp. Pi119AC and *Pantoea* sp. Pi72ED), commercial microbiological preparations (Totalhumus, Serenade, Polyversum, Contans), and hydro-thermotherapy on seed health and germination and development, physiological activity, and yielding of onion plants. The possibility of increasing the effectiveness of the treatments by adding whey and yeast to M2 or Totalhumus to M1 was also investigated.

2. MATERIAL AND METHODS

2.1. Plant material and treatments

The research was carried out on certificated onion seeds (*Allium cepa* L.) of the Density 4 Bio variety produced ecologically by Garden Seed and Nursery Stock Company TORSEED S.A. (Toruń, Poland, 53°00′49″N,18°35′53″E). Density 4 Bio has been a very popular and reliable variety for over 100 years. It is ideally suited to the cultivation in the climate of many countries and produces medium to large, globe-shaped, straw-colored bulbs with pure white flesh and a good flavor.

After purchase, the seeds collected at the recommended time and refined at TORSEED S.A. Company were stored at 10°C until treatment. Then, in mid-April, recommended for onion sowing, they were disinfected in the dark for 20 min in 1% potassium permanganate, dried to the initial moisture content at 20°C, and then soaked for 20 min at 20°C in the following biological agents:

a) natural remedies: whey applied according to the Mar-Rol recipe (Niewierz, Poland), Skotan yeast (*Yarrowia lipolytica*) applied according to the Skotan S.A. recipe (Chorzów, Poland);

b) commercial, biotechnical: Totalhumus, 0.3% (THE, Poland; containing humic and fulvic acids, salts of humic and fulvic acids, lignite extracts) dissolved in distilled water;

c) water suspensions of bacterial cells isolated by the authors from the rhizosphere soil and roots of onion and preserved in the SymbioBank at the Institute of Horticulture - National Research Institute, Poland:

– consortium M1 (*Pseudomonas* sp. Ps118AA and *Pantoea* sp. X58AD);

– consortium M2 (*Enterobacter* sp. Pi119AC and *Pantoea* sp. Pi72ED);

– M2 enriched with whey (1:1, v:v);

– M2 enriched with Skotan yeast (*Yarrowia lipolytica*) and whey (1:1:1, v:v:v);

– M1 with the addition of Totalhumus (1:1, v:v);

d) commercial microbiological biopreparations: Polyversum, 1.0% (Bio Agris, Poland; containing antagonistic fungus *Pythium oligandrum*), Serenade ASO, 0.5% (Bayer AG, Germany; containing strain QST 713 *Bacillus subtilis*) and Contans WG, 1.0% (Bayer CSB, Germany; containing parasitic fungus *Coniothyrium minitans*) all dissolved in distilled water;

e) hot water (50°C) as hydro-thermotherapy.

Seeds treated similarly with distilled water served as a control. The choice of these biological agents, the disinfection method, and the time and temperature of the seed treatment was based on the authors' previous research and literature data (Janas *et al*., 2005, 2019; Dorna *et al*., 2005; Sadowski *et al*., 2009). The bacteria collected in the SymbioBank and used in the experiment were isolated from the rhizosphere of representative soil and roots of onion plants on two organic farms in Poland. The density of their solution prepared for the treatment was as follows: *Pseudomonas* Ps118AA – 1.4 x 10^9 CFU ml^{-1}, *Pantoea* X58AD – 0.35 x 10^9 CFU ml^{-1}, *Enterobacter* Pi119AC – 2.2 x 10^9 CFU ml^{-1}, and *Pantoea* Pi72ED - 0.21 x 10^9 CFU ml^{-1}. The characterization of the obtained bacterial isolates was carried out by determining such properties as nitrogen fixation, IAA synthesis, production of siderophores, and solubilization of calcium phosphate $Ca_3(PO_4)_2$. Bacterial activity was assessed for the synthesis of indole acetic acid (culture in tryptone soy broth with 5mM l-tryptophan), availability of phosphorus compounds (culture on Pikovska medium for 10-14 days), synthesis of metabolites toxic to *V. dahliae* (culture on potato-glucose broth), and chitin degradation (broth with colloidal chitin) (Janas *et al*., 2020).

Identification of the isolated bacterial strains was based on the analysis of the 16S rRNA gene sequence and a comparison of the obtained sequences with NCBI (National Center for Biotechnology Information) data. https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome (Qian *et al*., 2009; Charbonneau *et al*., 2012).

The experiments were carried out in Central Poland (Skierniewice, 51°57′010″N, 20°08′030″E) in a greenhouse and an experimental ecological field, where the average minimum and maximum temperature varied during the experiments from 10 to 22°C in June, from 14 to 25°C in July, and from 14 to 25°C in August and the average monthly precipitation was 52, 57, and 43 mm, respectively. In the automatically ventilated greenhouse, the temperature varied from 18 to 30ºC and humidity from 50 to 80%, depending on sunshine. In the greenhouse, the plants were grown in 2 l pots (1 per pot) filled with a standard garden substrate (Klasmann TS1) and fertilized with YaraMila Complex (Yara) at a dose of 2 kg per 1 m^3 of substrate. The plants were watered with tap water as needed. In each experimental variant, 50 plants were grown in 3 replicates arranged in a random block system.

In the field, the plants were cultivated using the traditional method in an ecological system in podzolic soil where the previous crop was cereals. In each experimental variant, 50 plants were grown in three replicates arranged randomly at a spacing of 30 x 45 cm. The hydrothermal conditions depended on the weather.

2.2. Assessment of growth and physiological activity of plants

The treated seeds were stored in laboratory conditions (20ºC, 50% RH) for 24 h and then assessed in the following tests:

1) kinetics of germination and number of germinated seeds after sowing 3x100 seeds at 20ºC in Petri dishes (Ø 9 cm) on filter paper (grammage 250 m^{-2}) moistened with distilled water by daily counting the number of germinated embryos during 20 days (Janas *at al*., 2019);

2) dynamics of root and leaf growth in modified Phytotoxkit plates by daily measuring their lengths during 30 days (Romanowska-Duda *et al*., 2019);

3) dynamics of plant emergence by counting the number of emerging plants grown separately in 2-liter pots in the greenhouse every 2 days during 20 days (Grzesik *et al*., 2022);

4) dynamics of plant growth by assessing the height of leaves every 14 days throughout the vegetative period in the greenhouse and the field were the plants were cultivated at a 30x30 cm spacing (Grzesik *et al*., 2022);

5) gas exchange in growing plants (net photosynthesis, transpiration, stomatal conductivity, intercellular CO_2 content) measured with a TPS-2 apparatus (PP Systems, USA) in fully developed leaves in June and July in the field (Grzesik *et al*., 2022);

6) index of chlorophyll content measured in June and July with a Minolta SPAD-502 apparatus (Japan) in SPAD units and in fully developed leaves in the field (Grzesik *et al*., 2022);

7) identification of pathogenic microflora infecting the seeds (Janas *et al*., 2019);

8) bulb yields at the end of the cultivation period in the field (Grzesik *et al*., 2022).

The identification of the pathogenic microflora infecting the onion seeds was assessed 24 h after the treatment using an agar test. Seeds were placed in Petri dishes (Ø 9 cm, 10 seeds per dish) on solidified potato-dextrose (PDA) medium with the addition of streptomycin, which eliminates bacteria. They were then incubated for 10 days at 20ºC under alternating illumination with NUV light. The identification of fungal colonies was based on their appearance and sporulation using a high-sensitivity Leica microscope (Janas *et al*., 2019).

2.3. Statistical analysis

All the experiments concerning plant development and physiological activity were performed in triplicates of 50 plants each. A random block system was used in all the experiments in the laboratory, greenhouse, and field. The obtained results are reported as the mean ± standard deviation (SD) of three replicates. For each value, one-way ANOVA with Duncan's multiple range test was performed to determine the significance at the confidence limit of $p \leq 0.05$.

3. RESULTS

All the bacterial strains isolated from the rhizosphere soil and roots of onion, *i.e. Pantoea* sp. X58AD, *Pseudomonas* sp. Ps118AA, *Enterobacter* sp. Pi119AC, and *Pantoea* sp. Pi72ED, showed the capability of IAA synthesis, production of siderophores, and solubilization of calcium phosphate $Ca_3(PO_4)_2$. Additionally, the *Pantoea* sp. Pi72ED strain can also fix nitrogen (Table 1).

Table 1. Properties of bacterial strains isolated from the rhizosphere soil and roots of onion

Isolated strains	Nitrogen fixation	IAA synthesis	Siderophore production	$Ca_3(PO_4)_2$ solubilization
Pantoea sp. X58AD	-	+	+	+
Pseudomonas sp. Ps118AA	-	+	+	+
Enterobacter sp. Pi119AC	-	+	+	+
Pantoea sp. Pi72ED	+	+	+	+

Table 2. Identification of the isolated microorganism strains based on the similarity of the 16S rRNA gene sequence to the sequences stored in the NCBI database

Isolated bacteria strains	Genus/species of bacteria with the greatest similarity to the NCBI sequence	NCBI sequence No.	Degree of similarity (%)	Identified bacteria
X58AD	*Pantoea cypripedii* strain LMG 2657	NR_118394.1	98.81	*Pantoea* sp.
Ps118AA	*Pseudomonas silesiensis* strain A3	NR_156815.1	99.83	*Pseudomonas* sp.
Pi119AC	*Enterobacter ludwigii* strain EN-119	NR_042349.1	99.49	*Enterobacter* sp.
Pi72ED	*Pantoea cypripedii* strain LMG 2657	NR_118394.1	98.5	*Pantoea* sp.

Based on the analysis of the 16S rRNA gene sequence, the bacterial isolates chosen for experiments were found to belong to the genera *Pantoea* (X58AD, Pi72ED), *Pseudomonas* sp. (Ps118AA), and *Enterobacter* (Pi119AC) (Table 2). After identification, they were collected in the SymbioBank at the Institute of Horticulture – National Research Institute, Poland, and used to protect the plants against pathogenic microflora (Table 2).

The results of the mycological analyses of untreated commercial onion seeds indicate their poor health expressed by a high percentage of seeds infected with saprophytic and pathogenic microflora. Among the identified species, the fungus *Alternaria alternata* had the largest share in the total population. The pre-sowing seed treatment, regardless of the method, increased differently seed health and eliminated or significantly reduced the occurrence of *Alternaria alternata*, *Alternaria pori*, *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., *Stemphylium botryosum, Botrytis allii, Botrytis cinerea, Sclerotinia sclerotiorum, Stemphylium, Epicoccum purpurascen, Aspergillus* sp., and *Colletotrichum*. The greatest reduction in microflora infestation was achieved by the seed treatment with the bacterial consortiums from the SymbioBank, such as the water suspension of bacteria *Enterobacter* sp. Pi119AC and *Pantoea* sp. Pi72ED (M2) applied together with whey and yeast or the association of *Pantoea* sp. X58AD and *Pseudomonas* sp. Ps118AA (M1) used with Totalhumus. These treatments resulted in about four-fold lower levels of seed infection (70-100%) than in the control and about two-fold (40-100%) lower rates in comparison to the other treatments with biotechnical and microbiological applications (Table 3).

The pre-sowing onion seed treatments with both the bacterial consortiums (M1 and M2), whey, yeast, Totalhumus, Serenade, Polyversum, Contans, and thermo-hydrotherapy in hot water at 50°C had a spectacularly positive impact on the germination kinetics and number of germinated seeds (Fig. 1). The most effective in increasing the germination kinetics were the seed treatments with bacterial consortium M2 additionally enriched with whey and Skotan yeast or M1 with Totalhumus. The differences observed in the germination kinetics were most visible during 4-13 days after seed sowing. Eight days after sowing, the percentage of germinated seeds after the treatment was significantly higher than in the control, *e.g*. by 27% when M2 with whey and yeast was used and 19-23% after using the mixture of M1 with Totalhumus or the commercial preparations. However, after 19 days, the final number of germinated seeds treated before sowing with each of the biotechnical and microbiological methods was similar but significantly higher than in the control (Fig. 1).

All the seed treatments with the two bacterial consortiums (M1 and M2) as well as whey, yeast, biological agents, and hot water spectacularly increased the dynamics of growth of primary roots and leaves on water-moistened filter paper in the modified Phytotoxkit plates compared to the control, in which the seeds were soaked only in distilled water (Fig. 2). The bacterial consortiums exhibited similar (M1) and higher (M2) effectiveness in increasing root and leaf growth throughout the entire vegetation period, compared to the commercial preparations (Serenade, Contans, Polyversum, Totalhumus), and they all were more effective than the treatments with whey and hydrotherapy in hot water. The application of bacterial consortium M2 to the seeds together with whey and yeast was the most effective in the acceleration of roots and leaf growth. After 29 days from sowing the seeds, the length of roots and leaves increased by 46 and 57%, respectively, compared to the control, and by 23 and 26%, respectively, compared to the commercial preparations. In turn, the treatment of the seeds with microorganism association M1 and Totalhumus increased the length of roots and leaves at 29 days after sowing by 41 and 47%, respectively, compared to the control, and by 18 and 19%, compared to the commercial preparations (Fig. 2).

The dependencies between the seed treatment method and seedling growth in the Phytotoxkit plates were confirmed in the studies of the dynamics of emergence and growth of the plants in the greenhouse, where onion was cultured in 2-liter pots filled with the standard horticultural substrate (Figs 3, 4), and in the field on podzolic soil in weather-dependent conditions. In the field, plants obtained from seeds treated with the M2 consortium together with whey and yeast were 57% higher than the control and 19% higher than after using the commercial preparations. On the other hand, plants from seeds treated with M1 and Totalhumus were 53 and 16% higher, respectively (Fig. 5).

Table 3. Percent of seeds infected by microflora after treatment with biological agents in relation to all tested isolates and percentage of infected seeds

Seed-infecting microflora	Control	Whey	Yeast	M2	Whey+ M2	Whey+ Yeast+M2	Total-humus	M1	M1+Total-humus	Polyversum	Serenade	Contans	H_2O 50°C	$LSD_{0.05}$
Alternaria alternata	46.5 a* ±0.5	15.5 f ±0.4	17.0 d ±0.4	16.3 e ±0.3	12.0 h ±0.3	9.0 i ±0.3	24.0 c ±0.5	24.0 c ±0.5	14.3 g ±0.3	24.0 c ± 0.3	24.0 c ±0.3	23.5 c ±0.4	31.0 b ±0.4	0.6
Alternaria porri	4.2 a ±0.2	3.0 c ±0.1	2.5 d ±0.1	2.1 e ±0.2	2.0 e ±0.1	1.3 f ±0.1	2.5 d ±0.3	2.6 d ±0.2	1.8 e ±0.2	2.6 d ±0.1	2.5 d ±0.1	2.6 d ±0.1	3.5 b ±0.2	0.3
Aspergillus sp.	4.7 a ±0.3	2.0 d ±0.2	2.0 d ±0.2	2.0 d ±0.1	2.0 d ±0.1	1.4 e ±0.1	2.6 c ±0.2	2.6 c ±0.2	1.4 e ±0.1	2.5 c ±0.1	2.8 bc ±0.2	2.6 c ±0.1	3.0 b ±0.2	0.3
Botrytis alli	1.5 a ±0.1	0.5 d ±0.1	0.5 d ±0.1	0.5 d ±0.1	0.0 e ±0.0	0.0 e ±0.0	1.1 b ±0.1	1.0 bc ±0.1	0.0 e ±0.0	0.8 c ± 0.1	0.8c ± 0.1	0.8 c ±0.1	1.1 b ±0.1	0.2
Botrytis cinerea	2.2 a ±0.1	0.7 fg ±0.1	1.0 e ±0.1	1.0 e ±0.1	0.6 gh ±0.1	0.4 i ±0.1	1.6 c ±0.1	1.4 d ±0.1	0.8 f ±0.1	1.5 cd ±0.1	1.5 cd ±0.1	1.5 cd ±0.1	2.0 b ±0.1	0.1
Colletotrichum sp.	2 a ±0.1	0. 8 e ±0.1	0.6 f ±0.1	0.8 e ±0.1	0.0 g ±0.0	0.0 g ±0.0	0.0 d ±0.1	1.2 c ±0.1	0.0 g ±0.0	1.1 c ±0.1	1.2 c ±0.1	1.0 d ±0.1	1.4 b ±0.1	0.1
Cladosporium spp.	5.0 a ±0.2	1.0 d ±0.1	1.0 d ±0.2	1.0 d ±0.1	0.8 d ±0.1	0.5 e ±0.1	0.8 d ±0.2	1.2 c ±0.2	0.5 e ±0.1	1.1 c ±0.1	1.5 c ±0.2	1.5 c ±0.2	3.5 b ±0.1	0.2
Epicoccum purpurascens	4.5 a ±0.2	1.1 cd ±0.2	1.4 d ±0.1	1.4 d ±0.1	1.0 e ±0.1	0.0 f ±0.0	1.7 cd ±0.2	1.4 cd ±0.2	1.0 e ±0.2	1.8 c ±0.1	1.8 c ±0.1	1.8 c ±0.2	2.6 b ±0.2	0.3
Fusarium spp.	1.8 a ±0.2	1.2 c ±0.1	1.0 d ±0.1	0.5 e ±0.1	0.5 e ±0.1	0.0 g ±0.0	1.1 d ±0.1	1.0 d ±0.1	0.3 f ±0.1	1.0 d ±0.1	0.9 d ±0.1	1.0 d ±0.1	1.6 b ±0.1	0.1
Penicillium spp.	6.5 a ±0.2	1.0 h ±0.1	1.0 h ±0.2	1.7 f ±0.1	1.5 fg ±0.1	1.4 g ±0.1	2.2 de ±0.2	2.0 e ±0.2	1.6 fg ±0.1	2.4 cd ±0.2	1.3 cd ±0.1	2.5 c ±0.2	4.5 b ±0.2	0.2
Scerotinia sclerotiorum	2.0 a ±0.2	0.6 e ±0.1	0.8 de ±0.1	1.8 de ±0.2	0.4 f ±0.1	0.0 g ±0.0	1.1 c ±0.2	1.0 cd ±0.2	0.0 g ±0.0	1.1 c ±0.1	1.1 c ±0.1	1.1 c ±0.1	2.0 b ±0.1	0.2
Stemphylium botryosum	2.5 a ±0.2	1.0 cd ±0.2	1.0 cd ±0.1	1.0 cd ±0.1	0.9 d ±0.1	0.6 e ±0.1	1.1 cd ±0.2	1.0 cd ±0.1	0.6 e ±0.1	1.1 c ±0.1	1.2 c ±0.1	1.2 c ±0.1	1.9 b ±0.1	0.2
% of infested seeds	59.0 a ±2.9	22.0 de ±2.6	20.5 e ±1.9	20.5 e ±1.5	16.0 f ±1.8	12.5 g ±2.1	26.0 c ±2.5	26.3 c ±2.5	13.2 g ±2.1	24.0 cd ±2.4	26.0 c ±2.1	24.0 cd ±2.4	38.5 b ±2.4	2.5

M1 –consortium *Pantoea* sp. X58AD and *Pseudomonas* sp. Ps118AA, M2 – consortium of *Enterobacter* sp. Pi119AC and *Pantoea* sp. Pi72ED strains. *The means marked with the same letter, within particular seed-infecting microflora and percentage of infested seeds, are not significantly different, according to the Duncan multiple range test at a significance level of $p \leq 0.05$. The values are the mean ± SD of three replicates.

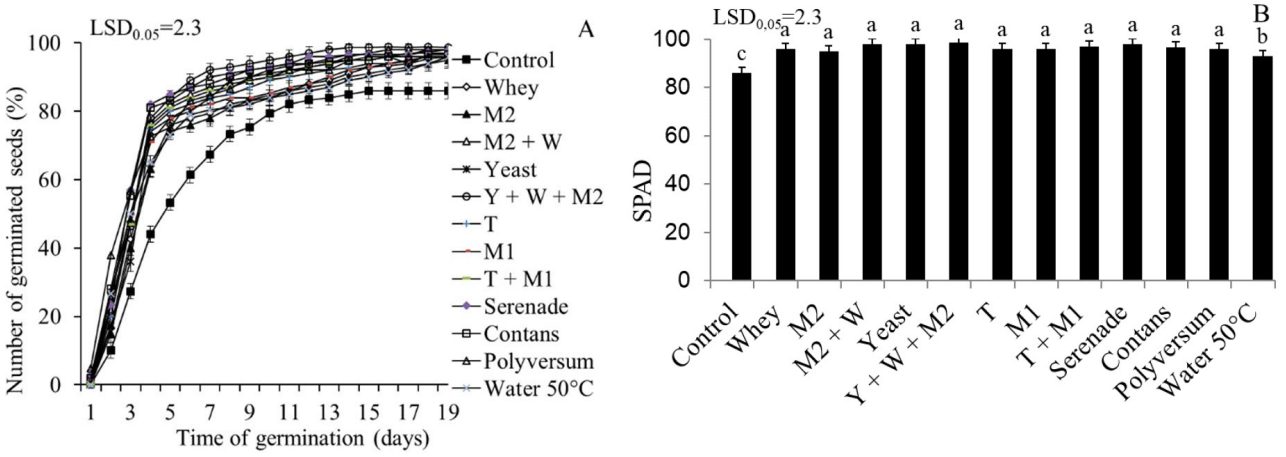

Fig. 1. Kinetics (A) and final number (B) of germinated onion seeds, treated with whey (W), Skotan yeast (Y), bacteria consortiums M1 and M2, commercial Totalhumus (T), Serenade, Contans, Polyversum and with water at 50°C. The means marked with the same letter are not significantly different, according to the Duncan multiple range test at a significance level of $p \leq 0.05$. Error bars show mean ± SD of three independent replicates.

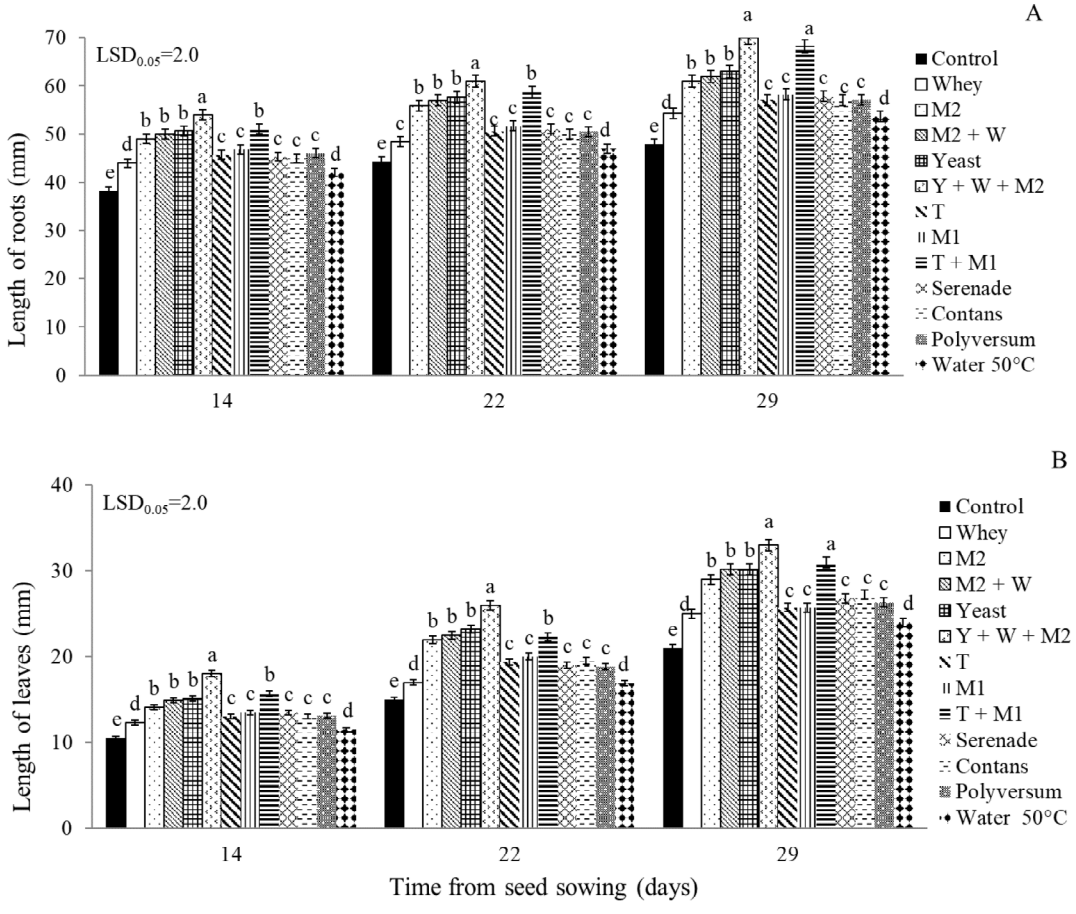

Fig. 2. Dynamics of root (A) and leaf (B) growth in modified Phytotoxkit plates, obtained from onion seeds treated with whey (W), Skotan yeast (Y), bacteria consortiums M1 and M2, commercial Totalhumus (T), Serenade, Contans, Polyversum and with water at 50°C. Statistical analysis, explained in Fig. 1, was done separately for each day after seed sowing.

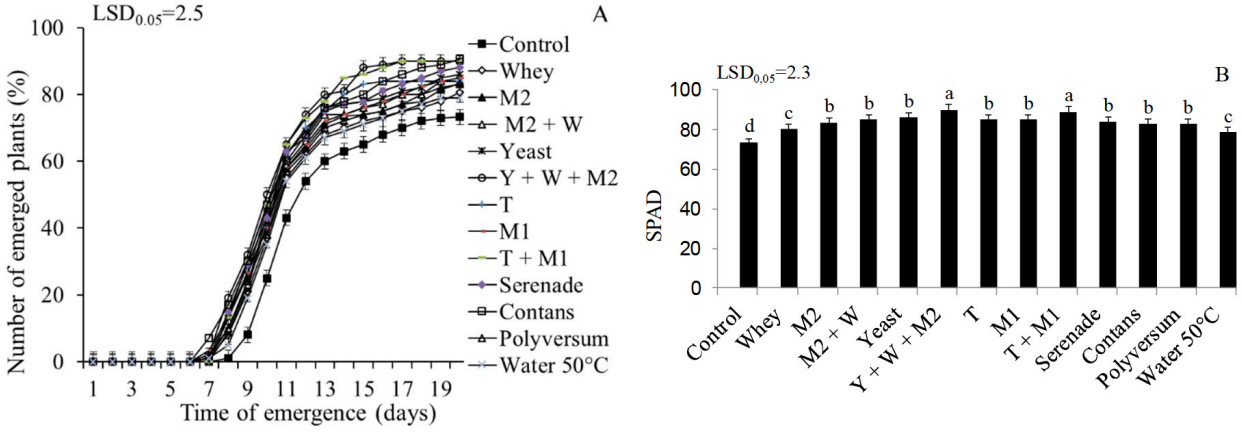

Fig. 3. Dynamics of emergence (A) and number of emerged plants (B) in greenhouse, obtained from onion seeds treated with whey (W), Skotan yeast (Y), bacteria consortiums M1 and M2, commercial Totalhumus (T), Serenade, Contans, Polyversum and with water at 50°C. Statistical analysis explanation in Fig. 1.

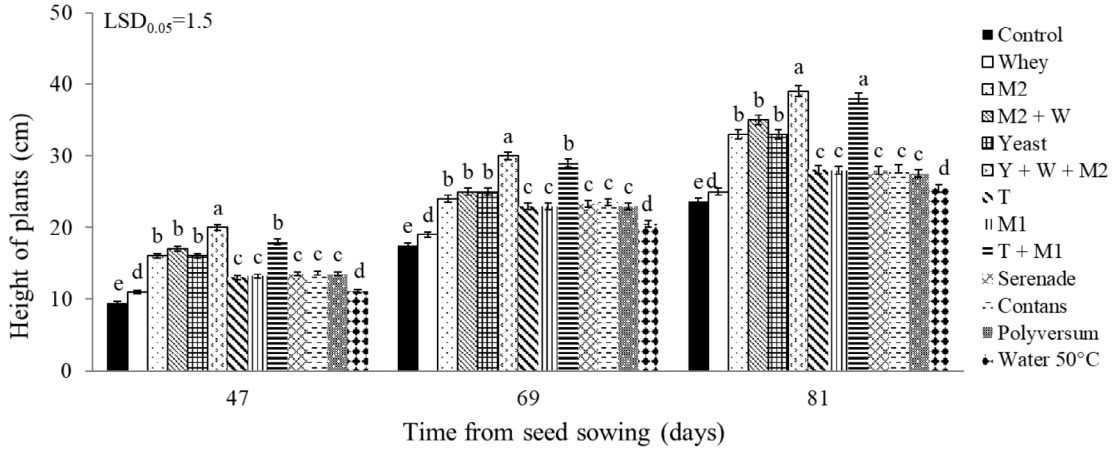

Fig. 4. Dynamics of plant growth in greenhouse, obtained from onion seeds treated with whey (W), Skotan yeast (Y), bacteria consortiums M1 and M2, commercial Totalhumus (T), Serenade, Contans, Polyversum and with water at 50°C. Statistical analysis, explained in Fig. 1, was done separately for each day after seed sowing.

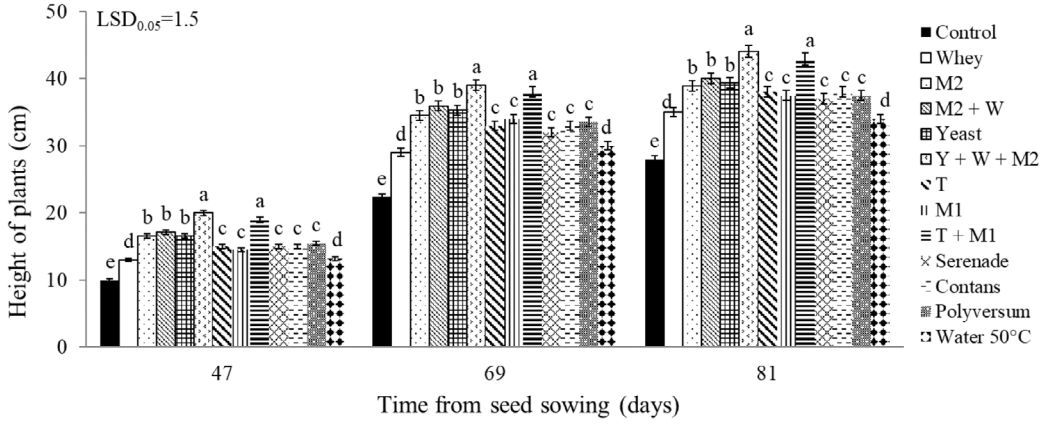

Fig. 5. Dynamics of plant growth in the field, obtained from onion seeds treated with whey (W), Skotan yeast (Y), bacteria consortiums M1 and M2, commercial Totalhumus (T), Serenade, Contans, Polyversum and with water at 50°C. Statistical analysis, explained in Fig. 1 was done separately for each day after seed sowing.

Table 4. Gas exchange and index of chlorophyll content in onion plants, obtained from seeds treated with whey, Skotan yeast, bacteria consortia M1 and M2, commercial Totalhumus (T), Serenade, Contans, Polyversum and with water at 50ºC. Averages from measurements in June and July

Treatment of seeds before sowing	Net photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{1})	Stomatal conductance (mmol H_2O^{-1} m^{-2} s^{-1})	Concentration intercellular CO_2 (µmol CO_2 air mol^{-1})	Index chlorophyll content (SPAD)
Control	1.85 f*±0.0.9	1.20 i±0.08	168 h±9.3	495 h±15.3	31.8 h±1.1
Whey	2.1 de±0.10	1.31 h±0.83	284 g±9.9	597 f±14.3	38.8 g±0.9
Yeast	2.3 c± 0.08	1.40 fg±0.08	302 f±7.7	623 d±13.1	48.1 e±0.8
M2	2.2 cd± 0.09	1.58 cd±0.06	320 de±9.3	613 de±13.7	50.6 cd±0.8
Whey + M2	2.5 b±0.07	1.62 c±0.06	385 b±9.4	632 c±12.9	51.9 b±0.6
Yeast + Whey + M2	2.7 a± 0.10	1.82 a±0.08	443 a±8.9	759 a±15.3	53.5 a±1.1
Totalhumus	2.1 de±0.08	1.49 gh±0.09	290 g±9.1	613 de±13.7	46.3 f±0.8
M1	2.2 cd±0.10	1.48 ef±0.08	320 de±9.3	611 de±13.5	50.6 cd±0.8
Totalhumus + M1	2.6 ab±0.11	1.71 b±0.07	368 c±9.6	675 b±14.3	52.4 ab±0.7
Serenade	2.3 c±0.07	1.52 de±0.07	320 de±9.3	604 ef±14.3	50.6 cd±0.8
Contans	2.3 c±0.07	1.59 cd±0.07	330 d±9.7	607 de±14.8	51.4 bc±0.7
Polyversum	2.2 cd±0.08	1.58 cd±0.06	311 ef±9.6	612 de±14.1	50.2 d±0.6
Water 50°C	2.0 e±0.09	1.39 gh±0.07	303 f±9.9	537 g±14.9	46.7 f±0.8
$LSD_{0.05}$	0.10	0.08	10.10	15.20	1.10

*Explanations as in Table 3.

Table 5. Yield of onion bulbs (kg 100 m^{-2}) obtained from seeds treated with whey, Skotan yeast, bacteria consortia M1 and M2, commercial Totalhumus (T), Serenade, Contans, Polyversum and with water at 50ºC

Seed treatment	Yield of bulbs
Control	470.8 k*±5.9
Whey	749.7 i±6.1
Yeast	800.1 e±6.0
M2	792 f±6.1
Whey + M2	830.1 c±6.0
Yeast + Whey + M2	861.6 a±5.9
Totalhumus	768.5 h±6.1
M1	783.6 g±5.6
Totalhumus + M1	843.5 b±6.0
Serenade	783.8 g±5.8
Contans	813.9 d±6.0
Polyversum	800.5 e±6.1
Water 50°C	698.2 j±5.9
$LSD_{0.05}$	6.2

*Explanations as in Table 3.

These positive results of all the seed treatments used were also confirmed by the increased gas exchange (net photosynthesis, transpiration, stomatal conductance, concentration of intercellular CO_2) and the index of chlorophyll content in the leaves measured in June and July (Table 4). The bacterial consortia M1 and M2 increased gas exchange and the index of chlorophyll content to a similar extent as the commercial preparations (Serenade, Contans, Polyversum, Totalhumus). The application of bacterial consortium M2 together with whey and yeast or M1 with Totalhumus was the most effective in increasing plant physiological activities (Table 4).

A consequence of the accelerated plant growth and intensification of their physiological activity was the increase in the bulb yield to an extent adequate to the demonstrated onion development parameters (Table 5). The seed dressing with all the applied biological agents resulted in a higher yield of healthy bulbs, compared to the control, in which the yield was significantly lower than in all the experimental variants. The bulb yield obtained from seeds treated with whey, yeast, and the M2 bacterial consortium was higher by 83% than in the control and by 79% than in the Totalhumus and M1 variant. In the case of hydrotherapy in water, the bulb yield was higher by 48% than in the control and by 59-72% than after the other treatments (Table 5).

4. DISCUSSION

The results of the presented research indicate a very low healthiness of commercially available organic seeds and the possibility to reduce microflora by using consortiums of antagonistic bacteria newly isolated from the onion rhizosphere and enriched with yeast, whey, or Totalhumus acting as physiological carriers of these microbes and additionally having fungicidal properties. It was found that the organic onion seeds were colonized by saprotrophic and pathogenic fungi, which are considered potentially dangerous since they can produce mycotoxins that reduce germination by as much as 20-35% and delay plant development (Dorna *et al*., 2005; Fernández *et al*., 2011; Medić-Pap *et al*., 2022).

The presented study has shown that effective protection of ecological onion seeds and plant growth stimulation can be achieved to varying degrees through the application of bio-products (whey, yeast), commercial preparations (Polyversum, Serenade, Contans, Totalhumus), hydrotherapy at 50ºC, and water suspensions of bacterial consortiums: *Pantoea* sp. X58AD and *Pseudomonas* sp. Ps118AA (M1) or *Enterobacter* sp. Pi119AC and *Pantoea* sp. Pi72ED (M2), newly isolated by the authors from the onion root rhizosphere. The research shows that these lately isolated consortium strains have similar (M1) and higher (M2) fungicidal and growth stimulating properties compared to the antagonistic fungus *Pythium oligandrum*, QST 713 *Bacillus subtilis*, and the parasitic fungus *Coniothyrium minitans* contained in commercial preparations Polyversum, Serenade, and Contans, respectively, as well as Totalhumus containing humic acids. An additional novelty of this research is the possibility of maximizing the protective and biostimulating properties of the isolated bacterial consortiums by additional treatment of seeds with whey, Skotan yeast, or Totalhumus having biocidal and microbe carrier properties.

Studies indicate that the positive impact of the isolated *Pantoea*, *Pseudomonas*, and *Enterobacter* strains on seeds and plant development was the result of their capability of IAA synthesis, solubilization of calcium phosphate ($Ca_3(PO_4)_2$), production of siderophores, and nitrogen-fixing abilities of *Pantoea* sp. Pi72ED (Sas-Paszt, 2022; Górnik *et al*., 2023). Among these key properties particularly noteworthy is the production of siderophores with a high affinity for several metal ions, mainly iron, and acting as carriers of antibacterial compounds introduced directly into the cell (Ignatowa *et al*., 2015; Yusuf *et al*., 2021). The increase in their fungicidal and growth stimulating effect was caused by yeasts, which alone can also fight plant phytopathogens and stimulate plant growth by competing for nutrients and space on the surface of plant organs, production of antibiotics and lytic enzymes, and excretion and induction of plant immunity by specific enzymes and production of phytohormones (Freimoser *et al*., 2019). In turn, whey, supporting isolated bacterial consortiums in their fungicidal and growth-stimulating capabilities, positively affects microbiological properties, increases microbial biomass, and enhances dehydrogenase and catalase activity (Akay and Sert, 2020).

Literature data on the activity of the tested bacteria *Pantoea* sp. X58AD, *Pseudomonas* sp. PS 118AA, or *Enterobacter* sp. in onions and the possibility to increase their biocidal effectiveness by biological agents has not been known so far, apart from the studies by Asselin *et al*. (2016), who identified only *Pantoea ananatis* and *Enterobacter* sp. occurring on plants of the studied species. The comparison of the effectiveness of the bacterial consortiums used with the previously studied genera *Trichoderma and Stachybotrys* as well as with the application of microorganisms (*Clonostachys rosea, Pseudomonas chlororaphis, Pseudomonas fluorescens*) needs additional research (Bennett *et al*., 2009; Abo-Elyousr *et al*., 2017; Cardiarelli *et al*., 2022). The problem is that the microorganisms used may have different modes of action or can provide different synergistic effects, causing a variable impact on seed sowing quality and plant development in diverse environments (Bennett *et al*., 2009; Cardarelli *et al*., 2022). The presented research addresses these problems and shows the positive effects of the demonstrated method on onion seed health and plant development in laboratory, greenhouse, and field conditions.

The study has shown that improvement of the health of onion seeds can also be achieved via thermohydrotherapy in hot water (50°C). The high efficiency of this easy-to-use treatment is related to the elimination of pathogens infecting the seed coat externally, which prevents their penetration into the seeds and damaging the embryo, and also by water removing germination inhibitors from the seed coat. However, in the case of other species, *e.g*. tomato cultivars, another temperature and treatment duration regime is needed (Divsalar *et al*., 2014).

5. CONCLUSIONS

Commercially available organic onion seeds are often of poor health as they are severely infected by saprotrophic and pathogenic micoflora, which cause a low germination rate and insufficient plant development. The presented study has demonstrated that seed infestation by these fungi can be differently reduced, while germination and plant development can be improved by a pre-sowing seed treatment with whey, yeast, commercial preparations (Polyversum, Serenade, Contans, Totalhumus), thermohydrotherapy at 50ºC, and water suspensions of bacterial consortiums: *Pantoea* sp. X58AD and *Pseudomonas* sp. Ps118AA (M1) or *Enterobacter* sp. Pi119AC and *Pantoea* sp. Pi 72ED (M2), newly isolated from the onion rhizosphere. These isolated strains have similar (M1) and higher (M2) fungicidal and growth stimulating properties as antagonistic microflora contained in commercial preparations Polyversum, Serenade, and Contans as well as

humic acids in Totalhumus. The protective and biostimulating properties of these isolated bacteria can be increased to a great extent by enrichment of the *Pantoea* sp. X58AD and *Pseudomonas* sp. Ps118AA consortium with whey and Skotan yeast or *Enterobacter* sp. Pi119AC and *Pantoea* sp. Pi72ED with the Totalhumus preparation.

Credit author statement:

Regina Janas: Conceptualization and methodology, analysis, investigation writing – original draft preparation. Lidia Sas-Paszt: Conceptualization and methodology of bacteria isolation. Mirosław Sitarek: Validation, reviewing paper. Paweł Trzciński: isolation from field and multiplication of bacteria. Anna Lisek: Identification of microorganism strains.

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