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NOVEL SOLID-PHASE MULTIBIORECYCLED BIOLOGICS BASED ON *Bacillus subtilis* AND *Trichoderma asperellum* AS EFFECTIVE POTATO PROTECTANTS AGAINST *Phytophthora* DISEASE

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Abstract

A total of 17 biologics based on the producer strains *Bacillus subtilis* and *Trichoderma asperellum* (=*T. harzianum*) are currently approved in Russia to protect potatoes from diseases. Great world experience has been gained in producing and use of traditional dry and liquid biologics. However, multirecycled industrial wastes as substrates for biologics are still not used anywhere in the world, and there is little information on effectiveness of formulations produced by industrial wastes' multistage biorecycling. This paper reports a successful experience of the sequential use of plant wastes as substrates for mushrooms and then for microbial strains to produce granular antifungal biologics. This is a relevant approach to biotechnologies for safer utilization of wastes as resources of cheap and affordable raw materials and their transformation into useful products. Our objective was to develop brand new multirecycled biologics based on plant pathogen antagonists and to estimate their efficacy. Plant wastes were converted to substrates for *B. subtilis* B-10 and *T. asperellum* T-36 producer strains by shiitake *Lentinula edodes* (Berk.) Pegler and oyster mushroom *Pleurotus ostreatus* (Jacq.: Fr.) P. Kummer HK-35 serial cultivation. The nutritional value of the obtained double biorecycled substrate, due to decomposition of cellulose and lignin of sawdust and wheat bran mixture by shiitake and oyster mushroom, was higher as compared to that of the initial substrate used for shiitake growing or of peat, a common solid-phase fermentation substrate. In particular, the protein content was higher (9.4±0.3 % vs. 2.7±0.3 % and 4.3±0.1 %, respectively), the nitrogen level was higher (1.5±0.3 % vs. 0.4±0.1 % and 0.6±0.1 %), and the C:N ratio reduced (38.3 vs. 81.2 and 92.9). Liquid microbial inoculums were cultured in standard Czapek (Biocompas-C Ltd., Russia) and corn-molasses (Carguil Ltd., Agroresource Ltd., Russia) nutrient media. Solid-phase fermentation of the double biorecycled lignin- and cellulose-containing substrate inoculated with 0.9×10^9 spores/ml *B. subtilis* B-10 and 2.8×10^{10} CFU/ml *T. asperellum* T-36 to produce the biologics took 10 days at 25-28 °C. The obtained biologics were tested on potato cv. Elizaveta in plot trials in the Leningrad Province (Producers' Cooperative Shushary, 2011). A reciprocally orthogonal scheme was used, and the plots were arranged in 4 replicates over 0.5 ha, with 10 m^2 test plot size and 482 plants sampled in total. A single application was performed at planting on May 12, 2011. The tubers were mixed with the biologics in the bunker of the potato-planting unit at a rate of 1 kg per 1.5 ton tubers (2 kg/ha). The basic potato growing technology included i) post-planting application of Sencor® herbicide (800 g/l, Bayer Crop Science, Germany); ii) post-germination double application (with one-week interval) of Terraflex® 17/17/17 inoculant (2.8 and 1.6 kg/ha, Nu3 N.V., Belgium); iii) post-germination single application of Aquadon micro inoculant (2.0 l/ha, Orgpolymersyntes, Russia), Extrasol® microbe fertilizer agent (2.0 l/ha, BisolbyInter Ltd., Russia), Zircon inoculant (10 g/ha, ANO Nest-M, Russia), herbicides Lazurite (0.5 l/ha, AO Avgust, Russia) and Titus™ (20 g/ha, DuPont, USA); and iv) treatments with fungicides after row closure as follows: Bravo® (1.5 l/ha, Syngenta AG, Switzerland) and Ridomil gold® (1.5 l/ha, Syngenta AG, Switzerland) in 2 weeks; Revus® (250 g/ha, Syngenta AG, Switzerland) in 4 weeks, and Shirlan® (0.4 l/ha, Syngen-

ta AG, Switzerland) in 6 weeks. The final fertilization with Terraflex® (2.8 kg/ha) combined with Shirlan® treatment (0.4 l/ha) were carried out 2 weeks before harvesting. The basic agrotechnology without biologics served as the control. Standard biometric and phytopathological indicators were used. The disease signs and biometric parameters were assessed in 3-week seedlings (1-2 leaf layer phase) and at row closure. Then two disease surveys were performed at the beginning and at the end of blooming, and final indicators for tubers were estimated at harvesting. Data processing by ANOVA and Student's *t*-test for pairwise comparison revealed that the biologics caused a significant increase in plant growth rate and the leaf area growth at the beginning of vegetation. The healthy tuber yield was 240 and 690 g/m^2 higher for *B. subtilis* B-10 and *T. asperellum* T-36 biologics, respectively, as compared to the control ($p \leq 0.10$). Due to the biologics, the late blight intensity was 7.2 times lower and 11.6 times lower, respectively ($p \leq 0.01$). The number of affected tubers, including those with signs of secondary bacterial infection, decreased almost 2 times, by 140 and 130 g/m^2, respectively ($p \leq 0,01$). Thus, solid plant waste multirecycling is a prospective way to produce granular environmentally safe biologics for plant protection against diseases. In the developed three-step technology, the wastes from edible mushroom double cultivation on sawdust mixed with wheat bran possess high nutritional value as a substrate for solid microbial cultures.

Keywords: multirecycled biologics, efficacy, potato, diseases, protection, microbial antagonists, multirecycling, *Bacillus subtilis*, *Trichoderma asperellum*

In 2019, 17 biologicals were permitted in Russia for potato protection against disease (the State Catalogue of Pesticides and Agrochemicals Permitted for Application in the Territory of the Russian Federation. Moscow, 2019). Most of them (13 preparations) are bacteria-based, 11 are products of liquid-phase culture of *Bacillus subtilis* (Ehren.) Cohn, and 3 products were obtained in liquid-phase or liquid-phase-solid-phase culture of *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg (= *Trichoderma harzianum*) strains [1]. These species and strains are characterized by a large variety of metabolic processes, are hardy when cultured and well-suited for intensive technologies, and also possess environmental plasticity [2-5]. Complex composition of their bioactive compounds with different action provides their bactericidal and fungicidal properties or hyperparasite activity (for *Trichoderma* spp.) [6-8). A number of microbial metabolites promote plant growth and development, and some are capable of increasing nonspecific plant resistance to diseases [9, 10]. The peculiarities of secondary metabolism of *B. subtilis* and *T. asperellum* strains determine multiple functionality of preparative form on their basis [11, 12].

Biological preparations are produced by different methods. Submerged culture technology is deemed more appropriate as controlled fermentation conditions provide a standardized final product. These technologies include concentration and drying steps which increase the costs, and moreover, the biological effects of these preparations require more time. The spore-forming bacteria are not picky in terms of cultivation conditions, and their spores sustain drying without loss of viability and biological activity. The micromycete strains that produce prolific biomass in submerged culture poorly form conidia in a liquid medium [18]. When fermented of on a solid substrate, micromycetes realize their potential of conidiogenesis to the fullest extent possible [13].

During production of biological preparations, solid-phase fermentation helps solve the problem of cheap and available raw materials for commercial biotechnologies via using plant waste of different related industries [14]. In biologicals obtained on plant substrates, producer strains for a long time remain viable both in the preparation and in soil after application [14]. The anthropogenic plant wastes of wood and timber industries, public utilities, forestry and agriculture containing lignocellulosic complex which is hard to recycle by most microorganisms are most efficiently used only in commercial edible mushroom growing [15-17]. Only xylotrophic basidial macromycetes are capable of decomposing lignocellulosic substrates and enriching low-value rough plant waste with fungal protein, easily digested carbohydrates, vitamins and mineral components,

thus providing an opportunity for usage of such substrates in various biotechnological processes [18-21]. After commercial mushroom growing completed and fruiting bodies are harvested, the substrate with penetrated mycelium can be used as feed additives, fertilizers or a substrate to grow other edible mushrooms and microorganisms with different target activity [22-24].

The usage of edible mushroom cultivation waste as a substrate containing cheap, accessible sources of nutrition and a mix of microelements required for fast growth and development of microorganism cultures is viewed as a promising way to produce biologicals [25, 26]. In more recent time the development of brand new multiple stage, non-waste, resource-saving and environmentally safe technologies of anthropogenic waste bioconversion based on higher basidial macromycetes and producer strains is of increasing interest (27). A wealth of experience has been accumulated globally in terms of production of liquid- and solid-phase fermentation on plant substrates (peat) and increased efficiency of commercial biological preparations based on production strains *B. subtilis* and *T. asperellum* [28, 29], including those used against potato diseases [30, 31]. However, it should be emphasized that multiconverted anthropogenic waste is not used anywhere in the world as a substrate for the production of biological products. There is lack of information about obtaining and efficiency of preparative forms developed based on multi-step (multi-)bioconversion of anthropogenic and agricultural waste [32].

This paper provides results of successful application of a brand new guided multiconversion of waste into useful products, and, specifically, of multiple usage of plant waste in commercial bioculture of edible mushrooms and production of granulated antifungal biological preparations. The multibioconversion of plant anthropogenic and agricultural waste was performed during sequential cultivation of *Lentinula edodes* (Berk.) Pegler (shiitake mushroom) and *Pleurotus ostreatus* (Jacq.: Fr.) P. Kummer HK-35 (oyster mushroom) mushrooms, and *Bacillus subtilis* B-10 and *Trichoderma asperellum* T-36 strains. The study shows increased nutrient value of converted waster compared to traditional substrates. Experimental samples of biological preparations against potato phytophthora rot improved plant protection in field test by 70-75% compared to the basic agrotechnical measures.

The purpose of the research is to develop a method of obtaining multiconversion biological preparations based on antagonists of plant pathogens and to evaluate the protective action of such biological preparations in potato farming.

Techniques. The collection strains of *Bacillus subtilis* B-10 and *Trichoderma asperellum* T-36 (National Collection of Microorganisms Pathogenic for Plants and their Pests; Innovation Technologies of Plant Protection Center for Collective Usage of Scientific Equipment, All-Russian Research Institute of Plant Protection; the collection was registered on January 28, 1998, No. 760 at the World Federation for Culture Collections, World Data Centre for Microorganisms — WFCC WDCM, Japan). Shiitake *Lentinula edodes* (Berk.) Pegler (summer hybrid) mycelium and oyster mushroom *Pleurotus ostreatus* (Jacq.: Fr.) P. Kummer HK-35 (shock-less hybrid) mycelium (Sylvan Hungaria Zrt., Hungary) were used for mushroom growing.

The K*LePo* (C3) substrate for subsequent solid-phase cultivation of microorganisms according to multibioconversion technology were obtained via double conversion of commercial substrate for shiitake П*Le* (C1) based on plant waste, the oak wood shavings (88.9%) and wheat siftings (10%) with addition of $CaCO_3$ (0.1%) and $CaSO_4 \cdot 2H_2O$ (1%) (by weight at 70% humidity). First, shiitake mushrooms were cultivated on sterilized C1 (П*Le*) for 3 months at 18-23 °C and 85-95% air humidity by semi-commercial small-volume submerged solid-phase method, resulting in fruiting bodies and spent substrate (C2). Then,

oyster mushrooms were cultivated on C2 for 2 months at 20-22 °C and 85-95% air humidity until the formation of fruiting bodies and C3 substrate completely penetrated by mycelia of these mushrooms. The composition of C3 (edible basidiomycete cultivation waste) was characterized based on its nutrition value (contents of polysaccharides, protein, total and amino nitrogen, amino acids, vitamins and microelements) by comparing with industrial substrate C1 (П*Le*) for shiitake cultivation and with lowland peat used in production of peat-based biologicals. The analyses were performed in Agrophysical Institute (St. Petersburg) in compliance with GOST GOST 26177-84 (Fibertec 8000 system), GOST 51417-99 (Digestor 2520 system), GOST 31675-2012 (Fibertec system) (all systems by Foss Tecator, Sweden); GOST 32903-2014 (Steyer liquid chromatograph, Aquilon, Russia); GOST 15962-2014 (atomic absorption spectrometer AA 240, Varian Techtron Pty Ltd, Australia); GOST 15607-2015 and GOST 34230-2017 (Steyer liquid chromatograph, Aquilon, Russia).

For inoculum (the first stage of biologicals production), *T. asperellum* T-36 was grown 5 days in liquid Czapek standard synthetic medium (2 g/l $NaNO_3$, 1 g/l KH_2PO_4, 0.5 g/l $MgSO_4$, 0.5 g/l KCl, 0.01 g/l $FeSO_4$, 20 g/l glucose; pH 7.0) (Biocompass-C, Russia) at 24-26 °C (250 rpm, New Brunswick™ Innova® 44 incubator shaker, Eppendorf, Germany). *B. subtilis* B-10 strain was cultured 3 days in optimized corn-molasses medium (30 g/l corn-steep extract, 15 g/l molasses; pH 7.8) (Cargill, Agroresurs LLC, Russia) at 27-28 °C (150 rpm, New Brunswick™ Innova® 44 incubator shaker). The titres of inoculums were determined by serial dilution procedure with plating onto agar-based media (Czapek agar, HiMedia Laboratories, India; dry nutrient agar SPA, Scientific and Production Association Microgen, Russia). The titers were 2.5×10^9 CFU/ml for *T. asperellum* T-36 and 3.8×10^{11} CFU/ml for *B. subtilis* B-10. In the second stage (solid-phase culture) C3 was inoculated with *T. asperellum* T-36 (2.8×10^{10} CFU/ml) and *B. subtilis* B-10 (0.9×10^9 spores per ml), and the strains were grown for 10 days at 25-28 °C in a thermostatically controlled chamber (laboratory thermostat PRO TC 30/120-500, Scientific and Production Association Prooborudivaniye, Russia).

The nutrient media and substrates were sterilized by autoclaving (5075ELVPV D, Tuttnauer Europe B.V., Netherlands).

The experimental samples of multiconversion biological preparations were tested in plot tests (Producers' Cooperative Shushary, Leningrad Province) on table purpose potato variety Elizaveta (medium early, high-yield, moderate resistant to phytophthora rot, cultivated in the Central, North-Caucasian, North-Western, Northern, Volga-Vyatka and Far Eastern region of Russia, released in the North-Western Region; originated by North-Western Agricultural Research Institute and Vsevolozhsky Plant Breeding Station).

The preparations were applied once during planting (May 12, 2011, in the morning at 17 °C and 72% relative air humidity). The tubers were mixed with biological preparations in a potato planting device bunker (1 kg per 1.5 tons of tubers, 2 kg per ha). The preparations were combined with basic agrotechnical and protective measures (in control group, the biologicals were excluded). The plot areas in test and control groups were 0.5 hectares each. The basic potato growing technology included i) post-planting application of Sencor® herbicide (800 g/l, Bayer Crop Science, Germany); ii) post-germination double application (with one-week interval) of Terraflex® 17/17/17 inoculant (2.8 and 1.6 kg/ha, Nu3 N.V., Belgium); iii) post-germination single application of Aquadon micro inoculant (2.0 l/ha, Orgpolymersyntes, Russia), Extrasol® microbe fertilizer agent (2.0 l/ha, BisolbyInter Ltd., Russia), Zircon inoculant (10 g/ha, ANO Nest-M, Russia), herbicides Lazurite (0.5 l/ha, AO Avgust**,** Russia) and Titus™ (20 g/ha, DuPont, USA)**;** and iv) treatments with fungicides

after row closure as follows: Bravo® (1.5 l/ha, Syngenta AG, Switzerland) and Ridomil gold® (1.5 l/ha, Syngenta AG, Switzerland) in 2 weeks; Revus® (250 g/ha, Syngenta AG, Switzerland) in 4 weeks, and Shirlan® (0.4 l/ha, Syngenta AG, Switzerland) in 6 weeks. The final fertilization with Terraflex® (2.8 kg/ha) combined with Shirlan® treatment (0.4 l/ha) were carried out 2 weeks before harvesting (September 5, 2011).

A reciprocally orthogonal scheme was used; the plots were arranged in 4 replicates over 0.5 ha, with 10 m^2 test plot size; 482 plants were sampled in total [33]. Standard plant biometric and phytopathologic parameters (plant growth rate, foliage, plant disease incidence and development, crop loss, absolute biological efficiency of the preparation and biological efficiency compared to control) [34] were used. Two biometric estibations and registration of onset of symptoms of the disease were performed on 3-4-week-old potato seedlings at the phase of leaf layers 1-2 and with closing of the rows on 6-7-week-old plants at the phase of leaf layers 9-10; two phytopathological records of onset of symptoms of the disease were performed at the start and end of blossoming (July 19 and August 16, 2011, respectively); one registration was performed during harvesting of tubers on September 5, 2011 [35]. Potato harvest was established using five randomly distributed 1 m^2 plots per variant in tests and control [33, 35].

The statistical processing with Microsoft Excel 2010 and Statistica 6.0 software (StatSoft, Inc., USA) included dispersion analysis (ANOVA), calculation of mean values (*M*), standard error of the mean (±SEM). In pair-wise comparison of variants the statistical significance of differences was analyzed based on Student's *t*-test.

Results. By comparing the composition of multiconversion waste after cultivation of edible basidiomycetes (C3 — K*LePo*) with the composition of multiconversion waste of commercial substrate for *L. edodes* cultivation (C1 — П*Le*), and of lowland peat (table 1) showed that substrate base of lab samples after multibioconvesion can be characterized as organic fertilizers containing available carbohydrates from decomposition of cellulose and lignin of C1 by basidiomycetes with the increased content of nitrogen and protein and a reduced C:N ratio (see Table 1).

1. Converted shiitake mushroom—oyster mushroom substrate (K*LePo*) **composition after sequential cultivation of *Lentinula edodes* and *Pleurotus ostreatus* on plant waste as compared to commercial substrate for shiitake mushrooms** (П*Le*) **and lowland peat** (*M*±SEM, semi-commercial cultivation)

Component	П*Le* [a]	K*LePo* [c]	Lowland peat [b]
Percent of absolute dry weight:			
cellulose	36.5±1.2*** (a/c)	16.7±0.3** (c/b)	15.6±0.3
lignin	24.3±3.4* (a/c)	17.3±0.5	22.1±0.4*** (b/c)
crude protein	2.7±0.3	9.4±0.3*** (c/b; c/a)	4.3±0.1
total nitrogen	0.4±0.1	1.5±0.3*** (c/b; c/a)	0.6±0.1
amino nitrogen	0.3±0.1	1.2±0.1*** (c/b; c/a)	0.1±0.02
ash	13.5±3.2*** (a/c)	2.5±1.6	3.7±0.3
Amount per absolute dry weight, ng/kg:			
essential amino acid	14.1±2.3	14.3±9.9	9.2±0.1
amino acid pool	32.4±10.3	31.4±16.7	22.9±0.1
Ca	1343.1±228.7	1465.4±351.7	2625.3±52.8*** (b/c)
Na	157.4±10.2	189.3±57.2	380.3±28.2*** (b/c)
K	3089.3±32.4	2643.5±321.2*** (c/b)	1250.1±28.9
Mn	454.1±89.6	581.8±185.6	360.8±85.4
Fe	329.6±59.8	278.3±87.2	208.8±21.1
biotin	0.03±0.01	0.04±0.01*** (c/b; c/a)	0.02±0.00
thiamine	0.3±0.1	0.5±0.1	0.6±0.0
riboflavin	1.7±0.3	4.3±0.1*** (c/b; c/a)	3.1±0.2
C:N	81.2	38.3	92.9

N o t e. Substrate description (П*Le*, K*LePo*, lowland peat) see in Techniques sction.
*, **, *** The differences between K*LePo* and П*Le* (c/a; a/c) and between K*LePo* and lowland peat (b/c; c/b) are statistically significant at $p \le 0.10$; $p \le 0.05$ and $p \le 0.01$, respectively.

Concentrations of nutrients for *T. asperellum* T-36 and *B. subtilis* B-10 growth on the shiitake mushroom—oyster mushroom converted substrate significantly ($p \leq 0.01$) exceeded the values for lowland peat which is commonly used as a solid-phase substrate in manufacturing biologicals. The total nitrogen and protein content was 2.0 times higher, microelements and vitamins were 1.5-2.0 times higher, and concentration of easily accessible carbohydrates was more than 1.5 times higher (see Table 1).

The titre of the multiconversion biologicals based on *T. asperellum* T-36 and *B. subtilis* B-10 in solid-phase culture on C3 (K*LePo*) was 10^{10} CFU/g.

During testing field efficiency of these preparations against potato phytophthora rot, an average monthly temperature and relative air humidity were as follows: 13.1 °C and 67.2 % in May; 19.8 °C and 70.1% in June; 24.4 °C and 68.5% in July; and 19.1 °C and 69.2% in August. In 2011, 42 sunny days were registered during plant vegetation. In 2011, field tests revealed significant growth acceleration ($p \leq 0.01$) and an increase in plant foliage by 1.2 times under the effect of both multiconversion biologicals as compared to the basic technology only (without use of biologicals) at the beginning of plant vegetation. The yield of healthy tubers as influenced by the preparations based on *B. subtilis* B-10 and *T. asperellum* T-36 strains significantly ($p \leq 0.10$) exceeded control, by 240 and 690 g/m^2, respectively (Table 2).

2. Development of potato plants (Elizaveta variety) as influenced by lab samples of multiconversion biologicals based on *Trichoderma asperellum* T-36 and *Bacillus subtilis* B-10 (*M*±SEM, Producers Cooperative Shushary, Leningrad Province, 2011)

Variant	Average plant growth rate, mm per day		Rate of foliage growth, leaf layers per day		Yield of healthy tubers, kg/m^2
	layer 1-2	layer 9-10	layer 1-2	layer 9-10	
BAPM + LO T-36 SHV, G	1.65±0.05**	10.61±0.32***	0.17±0.01***	0.25±0.01	5.12±0.32
BAPM+ LO B-10 SHV, G	1.51±0.04	12.71±0.27*	0.15±0.01	0.31±0.01***	5.57±0.43
BAPM (control)	1.49±0.04	12.09±0.36	0.15±0.01	0.26±0.01	4.88±0.32

N o t e. BAPM means basic agrotechnical and protective measures; LO T-36 SHV, G — laboratory samples of multiconversion biological preparations based on *T. asperellum* T-36, LO B-10 SHV, G — laboratory samples of multiconversion granulated biological preparation based on *B. subtilis* B-10 (samples were obtained using conversion shiitake—oyster mushroom substrate).

*, **, *** Differences with the control are statistically significant at $p \leq 0.10$; $p \leq 0.05$ and $p \leq 0.01$, respectively.

3. Phytophthora rot damage during blossom and weight of affected tubers in potato variety Elizaveta as influenced by lab samples of multiconversion biologicals based on *Trichoderma asperellum* T-36 and *Bacillus subtilis* B-10 (*M*±SEM, Producers Cooperative Shushary, Leningrad Province, 2011)

Variant	Phytophthora rot incidence, %		Phytophthora rot intensity,%		Weight of affected tubers, kg/m^2
	S	E	S	E	
BAPM + LO T-36 SHV, G	13.8±0.7*	16.1±0.6*	1.7±0.7*	3.6±0.9*	0.16±0.04*
BAPM+ LO B-10 SHV, G	14.9±0.4*	18.6±0.2*	2.1±0.3*	2.3±0.5*	0.17±0.03*
BAPM (control)	57.5±1.8	62.7±3.2	10.1±3.6	26.3±2.4	0.30±0.03

N o t e. S — start of blossoming, E — end of blossoming; BAPM means basic agrotechnical and protective measures; LO T-36 SHV, G — laboratory samples of multiconversion biological preparations based on *T. asperellum* T-36, LO B-10 SHV, G — laboratory samples of multiconversion granulated biological preparation based on *B. subtilis* B-10 (samples were obtained using conversion shiitake—oyster mushroom substrate).

* Differences with the control are statistically significant at $p \leq 0.01$.

The multiconversion biologicals based on *B. subtilis* B-10 and *T. asperellum* T-36 reduced the incidence of phytophthora rot 3.4- and 3.9-fold, respectively, and its development 7.2- and 11.6-fold, respectively ($p \leq 0.01$). In comparison with the control group the weight of affected tubers, including those showing signs of secondary bacterial infection, significantly ($p \leq 0.01$) decreased almost 2.0 times (by 140 and 130 g/m^2, respectively) (Table 3).

4. The efficiency of a single use of novel multiconversion biologicals based on *Trichoderma asperellum* T-36 and *Bacillus subtilis* B-10 against phytophthora rot (Elizaveta variety, Producers Cooperative Shushary, Leningrad Province, 2011)

Variant	Biological effectiveness, %	Biological effectiveness from control, %
BAPM + LO T-36 SHV, G	84.4	74.7
BAPM+ LO B-10 SHV, G	81.4	69.8
BAPM (control)	38.3	

N o t e. BAPM means basic agrotechnical and protective measures; LO T-36 SHV, G — laboratory samples of multiconversion biological preparations based on *T. asperellum* T-36, LO B-10 SHV, G — laboratory samples of multiconversion granulated biological preparation based on *B. subtilis* B-10 (samples were obtained using conversion shiitake—oyster mushroom substrate).

In our tests, the biological effectiveness of potato plant protection scheme which includes fertilizers and growth promoters, herbicides, and multiple applications of chemical fungicides did not reach 40% (Table 4). Low efficiency of conventional measures is due to the increasing resistance of pathogens to chemical pesticides during the recent years as a result of anthropogenic transformation of agricultural ecosystems and their phytosanitary deterioration [1, 26, 31].

The multiconversion biologicals that we suggest increased total biological efficiency of basic protective measures by more than 2.2 times (see Table 4). Given the agrotechnical measures and protective chemical treatments used, the efficiency of multiconversion preparations based on *T. asperellum* T-36 and *B. subtilis* B-10 was rather high, 74.7 and 69.8%, respectively (see Table 4).

Most biological preparations based on *B. subtilis* and *T. asperellum* recommended in Russia for potato plant protection against disease during vegetation have titres 10^9-10^{11} CFU/g and 10^8-10^{10} CFU/ml respectively depending on their preparative forms [1, 12]. In the suggested novel multiconversion biologicals the titre is 10^{10} CFU/g which corresponds to analogs, e.g. Alirin B, TAB; Alirin B, SP; Alirin B, Zh; Gliocladin, TAB; Gliocladin, SK; Gliocladin, SP; Gliocladin, Zh; Trihozin, SP (all produced by Management Company ABT-Group and All-Russian Institute of Plant Protection, Russia) [30, 32]. It is a known fact that waste substrates resulting from commercial cultivation of edible mushrooms are successfully used in agriculture as organic fertilizers [36, 37]. Among other things, such waste show good results when used as fertilizer and growth promoter during potato farming [38]. In our report, the substrate resulted from a two-stage biological conversion of cellulose- and lignin-containing industrial and agricultural wastes by edible basidiomycetes (shiitake mushroom and oyster mushrooms) is an actual organic fertilizer enriched with microelements and vitamins [25, 36]. An increase in biometric parameters of potato plants and tuber yield emphasized in this study, as well as improvement of traditional integrated potato protection offer prospects for development of protective biologicals which additionally possess the properties of biofertilizers and biostimulants [39-43]. The biologicals we propose in this paper, when used in field conditions at a suggested dosage, ensured reliable protective effect corresponding to that described by global developers for similar formulations [44-47].

Overall, our findings show a possibility to produce most suitable for soil application and environmentally safe granulated biologicals using multibioconversion of plant industrial waste by edible mushrooms and then by microorganisms producing bioactive metabolites. The new data have been obtained about nutrient value of the substrates, resulted from cultivation of edible mushrooms, when these substrates are used in solid-phase culture as growth media for strains producing biological preparations. One application of novel multiconversion biologicals based on *Bacillus subtilis* B-10 and *Trichoderma asperellum* T-36 is proven to increase the efficiency of generally accepted basic potato protection measures against phytophthora rot by 70 and 75%, respectively.

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